Institutionen för medicinsk epidemiologi och biostatistik

Diet in Epidemiology – Assessment, Validity and Association with Upper Respiratory Tract Infection

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ABSTRACT

This thesis covers the evaluation of two new interactive web- and meal-based food frequency questionnaires (FFQ). In addition, it investigates the potential association between dietary intake as well as physical activity and the susceptibility to upper respiratory tract infection (URTI).

In **Paper I** and **II**, the validity of Meal-Q and MiniMeal-Q as well as the reproducibility of Meal-Q was evaluated among 163 participants in the validation study VALMA. MiniMeal-Q is a shorter version of Meal-Q, including about 30% less food items. As reference methods, we used 7-day weighed food records (WFR) for energy and nutrients and doubly labeled water for energy expenditure. Evaluating ranking ability with the WFR, Meal-Q and MiniMeal-Q classified 69-90% and 67-89% of the participants into the same/adjacent quartile for energy, macro- and micronutrients and fiber, respectively. The corresponding proportion with the doubly labeled water was 77%. The correlation coefficients with the WFR ranged r=0.33-0.74 for macronutrients and r=0.25-0.69 for micronutrients and fiber, and was r=0.18 for energy. Correlations with the doubly labeled water were r=0.42 for Meal-Q and r=0.38 for MiniMeal-Q. Bland-Altman agreement plots with the WFR showed on average large variances and trends of increasing underestimation with increasing intakes. Regarding reproducibility, the intra-class correlations for Meal-Q ranged r=0.57-0.90 for energy and macronutrients and r=0.50-0.76 for micronutrients and fiber. The results were in line with previous validation studies on FFQs. Furthermore, both Meal-Q and MiniMeal-Q had a short answering time of 17 and 7 minutes, respectively and were rated as highly user-friendly by the participants.

In **Paper III**, we evaluated the adherence to the Nordic Nutrition Recommendations (NNR) as a measure of a healthy diet and susceptibility to URTI. In a prospective cohort study of four months among 1,509 participants aged 20-60 years, diet was assessed with a web-based FFQ and URTI was self-reported in five follow-up questionnaires. We found no association between overall adherence to the NNR and URTI. However, high physical activity was associated with an 18% reduced risk of URTI (incidence rate ratio (IRR) 0.82, 95% CI 0.69-0.97).

In **Paper IV**, we investigated the association between intake of antioxidants and polyunsaturated fatty acids and URTI. In a prospective cohort study among 1,533 participants aged 25-64 years, participants reported URTI events on their own initiative by phone or a web-based form during 9 months of follow-up. Diet was assessed with MiniMeal-Q. We found that high dietary intake of vitamin C (IRR 0.69 (0.55-0.88)), vitamin E (IRR 0.77 (0.62-0.96)) and docosahexaenoic acid (DHA) (IRR 0.57 (0.39-0.83)) was associated with a reduced risk of URTI among women. No inverse association could be found among men, instead an increased risk of URTI was found for medium intake of vitamin E (IRR 1.42 (1.09-1.85)) and high intake of zinc (IRR 1.50 (1.04-2.16)) from food.

In conclusion, in **Paper I** and **II** we show that Meal-Q and MiniMeal-Q are two user-friendly FFQs with short answering time and good ranking ability of most nutrients. In **Paper III**, we found that high physical activity reduced the risk of URTI. Moreover, in **Paper IV**, high dietary intake of vitamin C, vitamin E and DHA was associated with a reduced risk of URTI among women. In contrast, medium vitamin E and high zinc intake from food was associated with an increased risk of URTI among men.

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"If we could give every individual the right amount of nourishment
and exercise, not too little and not too much, we would
have found the safest way to health."
—Hippocrates c. 460—377 B.C.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Denna avhandling omfattar en utvärdering av två nyutvecklade kostenkäter med avseende på validitet och reproducerbarhet. Avhandlingen inkluderar också studier på kost och motion och risken för att insjukna i övre luftvägsinfektioner.

Inom epidemiologisk forskning används ofta enkäter för att mäta kostintaget. En kostenkät består vanligtvis av en lista med livsmedel och drycker samt frågor om hur ofta man konsumerar dessa. Under åren 2008-2009 utvecklade vår forskargrupp två web-baserade kostenkäter, Meal-Q och MiniMeal-Q, med syfte att användas i epidemiologiska studier med web-baserad datainsamling. Eftersom mer än 90% av Sveriges vuxna befolkning har tillgång till Internet är web-baserade enkäter ett kostnadseffektivt sätt att samla in data på. Frågorna i Meal-Q and MiniMeal-Q är grupperade efter dagens måltider för att underlätta för deltagarna att minnas vad de äter. Båda enkäter är interaktiva, vilket betyder att de har uppföljningsfrågor som anpassas efter hur man svarat på tidigare frågor. På så sätt blir frågorna mer relevanta för deltagarna och svarstiden kortare.

Det är av stor vikt att utvärdera en enkäts validitet och reproducerbarhet. Därför genomförde vi en valideringsstudie under våren 2009 bland 163 män och kvinnor. Vi utvärderade validiteten av Meal-Q och MiniMeal-Q genom att jämföra dem med en 7-dagars vägd kostdagbok. Deltagarna blev instruerade i att väga all mat och dryck så noggrant som möjligt och föra i det i dagboken. Som komplement till kostdagboken använde vi dubbelmärkt vatten som mäter kroppens totala energiförbrukning. Den totala energiförbrukningen användes för att utvärdera energiintaget mätt med kostenkäterna. För att utvärdera reproducerbarheten av Meal-Q fick studiedeltagarna fylla i enkäten två gånger med tre veckors mellanrum.

I jämförelsen med kostdagboken fann vi att både Meal-Q och MiniMeal-Q underskattade intaget av energi och de flesta näringsämnen. Det dubbelmärkta vattnet visade också att energiintaget var underskattat i båda enkäter. Trots detta lyckades enkäterna rangordna deltagarna väl med avseende på om de åt lite eller mycket av ett näringsämne. Inom epidemiologisk forskning är det oftast av större vikt att kunna rangordna deltagarna med avseende på deras näringsintag än att kunna mäta det absoluta intaget. Ur denna synpunkt visade sig både Meal-Q och MiniMeal-Q mäta kosten på ett tillfredställande sätt för de flesta näringsämnen. Reproducerbarheten av Meal-Q var god. Meal-Q och MiniMeal-Q tog i genomsnitt 17 respektive 7 minuter att fylla i. Det är korta svarstider jämfört med många andra kostenkäter som vanligtvis tar mellan 15-30 minuter. Båda enkäter fick också högt användarbetyg av deltagarna.

Övre luftvägsinfektioner, dvs. förkylning och influensa, är en av de vanligast förekommande sjukdomarna i befolkningen och de årliga kostnaderna i förlorade arbetstimmar i USA är beräknade till mer än $20 miljarder. Trots detta vet vi väldigt lite om hur vi kan minska mottagligheten för infektioner. I två

kohortstudier har vi därför studerat ifall kost och motion kan påverka risken för övre luftvägsinfektioner.

I den första studien ville vi utvärdera ifall följsamhet till de Nordiska Näringsrekommendationerna (NNR) påverkade risken för övre luftvägsinfektioner. NNR ger rekommendationer om kostens sammansättning gällande kolhydrater, fett och protein samt rekommenderat intag av vitaminer och mineraler. Sedan 2004 innehåller NNR även rekommendationer om fysisk aktivitet. NNR har till syfte att främja en god hälsa och att minska kostrelaterade sjukdomar i befolkningen.

I januari 2004 lät vi 1,509 kvinnor och män fylla i en web-baserad enkät om kost och fysisk aktivitet. Under de följande fyra månaderna fram till maj fick deltagarna rapportera ifall de fått infektioner eller inte. Detta gjordes via web-enkäter som skickades ut vid fem tillfällen. För att utvärdera följsamheten till NNR skapade vi en modell med poängsättning för de olika rekommendationerna. Vi utvärderade dels hur väl man följde NNR i sin helhet, och dels hur man följde de individuella rekommendationerna. Resultaten visade inte på någon association mellan hög följsamhet till NNR i sin helhet och risk för infektion. Vi fann dock att deltagare som hade en hög fysiskt aktivitetsnivå hade en 18% minskad risk för infektioner.

I den andra kohortstudien undersökte vi om intaget av antioxidanter och fleromättade fettsyror påverkar risken för övre luftvägsinfektioner. Man har i tidigare studier funnit att antioxidanter spelar en viktig roll för immunförsvaret eftersom de skyddar mot fria radikaler som kan orsaka skada hos immunceller. Några av dessa antioxidanter är C-vitamin, E-vitamin, selen och zink. Fleromättade fettsyror har också visat sig påverka vårt immunförsvar genom att vara involverade i inflammatoriska processer. Kosttillskott av C-vitamin, E-vitamin och zink har i tidigare studier visat sig förebygga övre luftvägsinfektioner samt mildra symptomen.

Under sommaren 2011 rekryterade vi 1,533 män och kvinnor till att delta i studien. De fick fylla i kostenkäten MiniMeal-Q och ombads att via telefon alternativt ett web-formulär rapportera närhelst de fick en infektion. Studien pågick från september 2011 till maj 2012 och täckte således den huvudsakliga infektionssäsongen för övre luftvägsinfektioner i Sverige. Genom att rangordna deltagarna med avseende på deras intag av antioxidanter och fleromättade fettsyror kunde vi beräkna risken för att få en infektion bland de med högt intag jämfört med de med lågt intag av näringsämnena. Vi fann att ett högt intag av C-vitamin, E-vitamin och den fleromättade fettsyran DHA var kopplat till en minskad risk för infektion bland kvinnor med 31, 23 respektive 43%. Vi kunde inte se något sådant skyddande samband för män. Vi såg istället att ett medelhögt intag av E-vitamin och ett högt intag av zink var kopplat till en ökad risk för infektioner med 42 respektive 50%.

LIST OF PUBLICATIONS

I. Christensen SE, Möller E, Bonn SE, Ploner A, Wright A, Sjölander A, Bälter O, Lissner L, Bälter K. Two New Meal- and Web-Based Interactive Food Frequency Questionnaires: Validation of Energy and Macronutrient Intake. *Journal of Medical Internet Research*. 2013;15:e109.

II. Christensen SE, Möller E, Bonn SE, Ploner A, Bälter O, Lissner L, Bälter K. Relative validity of micronutrient and fiber intake assessed with two new interactive meal- and web-based food frequency questionnaires. *Journal of Medical Internet Research*. doi:10.2196/jmir.2965, http://dx.doi.org/10.2196/jmir.2965. *In press*.

III. Fondell E, Christensen SE, Bälter O, Bälter K. Adherence to the Nordic Nutrition Recommendations as a measure of a healthy diet and upper respiratory tract infection. *Public Health Nutrition*. 2010;14:860-9.

IV. Christensen SE, Plymoth A, Ploner A, Nyrén O, Bälter O, Bonn SE, Fondell E, Bälter K. Dietary intake and supplement use of vitamin C, vitamin E, selenium, zinc and polyunsaturated fatty acids and upper respiratory tract infection: a prospective cohort study. *Manuscript*.

RELATED PUBLICATIONS

I. Bonn SE, Trolle Lagerros Y, Christensen SE, Möller E, Wright A, Sjölander A, Bälter K. Active-Q: Validation of the Web-Based Physical Activity Questionnaire Using Doubly Labeled Water. *Journal of Medical Internet Research*. 2012;14:e29.

CONTENTS

LIST OF ABBREVIATIONS

AA	Arachidonic acid
BMI	Body mass index
DAG	Directed acyclic graph
DHA	Docosahexaenoic acid
DLW	Doubly labeled water
EPA	Eicosapentaenoic acid
FFQ	Food frequency questionnaire
GEE	Generalized estimating equations
ICC	Intra-class correlation
IVRS	Interactive voice response service
IQR	Interquartile range
MET	Metabolic equivalent task
NNR	Nordic Nutrition Recommendations
PAL	Physical activity level
PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
URTI	Upper respiratory tract infection
WFR	Weighed food record

1 INTRODUCTION

Through dietary intake, we are exposed to a large number of nutrients with the potential in enhancing or worsening our health. With advancements in research, we have today been able to link dietary intake to many diseases, such as cardiovascular disease, diabetes and several cancer forms. We have also learned that an adequate nutrient intake is essential for a functioning immune system[1].

When conducting research in nutritional epidemiology, the aim is to evaluate the association between dietary intake and various health outcomes. Due to the large variety of consumed food products, their complexity in nutrient composition as well as the difficulty in capturing true intake through self-reports, a correct estimation of exposure is a challenge. Nevertheless, it is essential to attempt to assess nutrient intake as correctly as possible to continue exploring potential associations between diet and health. The most commonly used method for assessing dietary intake in nutritional epidemiology is the food frequency questionnaire (FFQ). Is it cost-efficient and has relatively low participant burden. It generally assesses habitual dietary intake and is suitable for ranking individuals according to their nutrient intake. As with any method, it is important to evaluate its accuracy. In a validation study, one can estimate the validity and precision, and thereby quantify the amount of systematic and random error inherent in the assessment.

With recent increased research in nutritional immunology, a greater knowledge has emerged of how nutrients affect our immune system. We know today that a deficiency of almost any nutrient can hamper the immune function[1]. A major role of the immune system is to prevent its host from contracting infections. The most common illnesses among humans is acute upper respiratory tract infection (URTI)[2] and the American annual costs for work loss due to URTI are estimated to be more than $20 billion[3]. In this way, URTI contributes to both individual and societal economic burden. Nevertheless, we still know little about how to reduce the susceptibility to URTI. However, we know that the immune system is particularly sensitive to oxidative stress[4]. Here, nutrients with antioxidative properties, such as vitamin C, play a key role in protecting the function of the immune cells[5]. Polyunsaturated fatty acids (PUFAs) that affect inflammatory processes are further examples of nutrients involved in the immune system[6]. Another lifestyle factor affecting the immune function is physical activity and physically active individuals have been found to have a reduced risk of contracting URTI[7-10].

This thesis covers methodological considerations of dietary assessment in nutritional epidemiology with focus on validity and reproducibility of FFQs. In addition, it attempts to bring new knowledge into the field of nutritional immunology, more specifically dietary intake and URTI.

2 BACKGROUND

This chapter gives an overview of different dietary assessment methods and their accuracy as well as a brief description of nutritional immunology including URTI and dietary factors.

2.1 DIETARY ASSESSMENT METHODS

Among the more commonly used dietary assessment methods in epidemiological studies are FFQs, 24-hour recalls and food records. The FFQ comprises a list of food items and asks about the frequency of consumption during a specified time period to assess habitual dietary intake. Estimation of amounts is often made with standard portions. However, with the inclusion of multiple portion size choices, the FFQ can become semi-quantitative. With the 24-hour recall method, study participants are asked to report their intake of foods and beverages during the preceding 24 hours. It can be interview-based or self-assessed and the participants are asked to estimate the amount of food consumed. When using the food record, the study participants prospectively fill in a diary of their food and beverage consumption for a specified time period with the amount of food estimated or weighed.

Even if the 24-hour recall and the food record assess actual dietary intake, a single recall or food record does not say anything about the habitual dietary intake for that person. Hence, multiple recalls or food records spread out over a longer time period are needed to describe an individual's usual dietary intake. The FFQ can on the other hand assess habitual dietary intake over a specific time period and is usually used for this purpose. This enables researchers to rank individuals according to their level of nutrient intake. Ranking of dietary intake is usually of main interest in epidemiological studies rather than assessment of absolute intake[11].

The food list in the FFQ can have different formats, e.g. being food group-based where food items are grouped into categories such as dairy products, cereal products and meat products. The food list can also have a meal-based structure with food items grouped into the commonly consumed meals during a day. A meal-based structure has the advantage of helping the participant recall their dietary intake since the design mimics the daily eating pattern. Meal-based structures have also been shown to facilitate dietary recall in previous studies[12,13].

Since the aim with a FFQ usually is to rank individuals according to their nutrient intake, the design should capture both commonly and less commonly consumed food items in order to obtain a wide distribution of intake in a study population. For example, some food items are eaten less frequently but represent important

sources for certain nutrients, e.g. liver paste and black pudding that are important sources of iron. For an adequate estimation of nutrient intake, it is also crucial to have access to an extensive Food Composition Table with data on nutrient content of foods. As compared to the 24-hour recall and the food record, the FFQ puts little burden on the participant since the diet normally is assessed once, or with long periods in between, and it usually only takes 15-30 minutes to complete. The method is also cost-efficient and easy to administer and thereby enables assessment in large studies.

Collection of dietary data through FFQs has traditionally been done with printed questionnaires. However, with increased use of the Internet, web-based FFQs have become more common. In Sweden, more than 90% of the adult population has access to the Internet[14], which promotes the use of web-based FFQs in Swedish epidemiological studies. A web-based FFQ has many advantages over a paper-based version. It facilitates instant dissemination to a large number of study participants and data is directly collected in digital format ready to be linked to a nutrient database to obtain nutrient intakes. The interactive potential of the web-format also enables an automatic check of missing answers, which minimizes error and increases the data quality[15,16]. Moreover, an interactive design can adapt follow-up questions to previous answers and thereby make the questions seem more relevant to the participant as well as to keep the answering time short. Furthermore, interactive web-questionnaires have previously shown high compliance in a Swedish population with widespread Internet access[17].

2.2 ACCURACY OF DIETARY ASSESSMENT

The accuracy of a dietary assessment method is to which extent it describes the true dietary intake. Accuracy therefore implies both *validity* and *precision* (**Figure 1**), i.e. the lack of both systematic and random error.

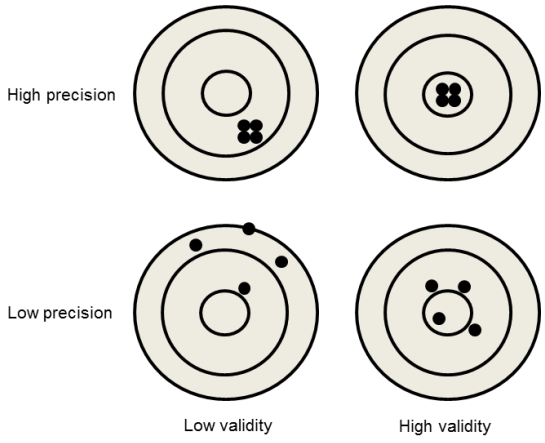

Figure 1. Illustration on validity and precision.

Assessing habitual dietary intake with high accuracy is a challenge. It requires a well-designed assessment method that is comprehensive, yet still attractive and easy to use in order to aid the participants in recalling their dietary intake. Besides the design of the assessment method, the accuracy is also dependent on the correctness with which the respondents report their intake. A crucial factor is the reliance on memory when reporting food intake retrospectively. Furthermore, *social desirability*, i.e. reporting food intake according to a current *norm*, is an issue that affects any type of method. The classic example is the under-reporting of unhealthy foods and over-reporting of healthy foods.

2.2.1 Validity of dietary assessment – lack of systematic errors

If a dietary assessment method assesses dietary intake with high validity, the assessment has low amounts of systematic errors. An assessment can have good internal validity, which means that the results are valid for the specific group they are obtained from. If the assessment also has good external validity, the results are also valid, or generalizable, to individuals outside the specific group. Internal validity is necessary for, but does not imply, external validity.

Systematic errors in dietary assessment can arise from different sources, being either related to selection bias or classification bias. When a study relies on volunteer participation, self-selection bias can be an issue. The aforementioned *social desirability* in reporting food intake can yield systematic under- and over-reporting of certain foods, which leads to misclassification. A limitation in the number of food items included in the FFQ can contribute to misclassification, i.e. we can only assess what is asked for. Moreover, the number of intake frequencies can also lead to misclassification of true intake.

2.2.2 Reproducibility of dietary assessment – lack of random errors

High reproducibility, or precision, of a dietary assessment method implies low amounts of random errors. The reproducibility is evaluated using repeated assessments of the dietary intake. True variation in dietary intake over time can affect the reproducibility and should be taken into consideration. However, true within-subject variation and random error are difficult to disentangle. It is therefore crucial to choose a proper time period for the repeated assessment(s).

Random errors are errors that occur due to chance alone. The presence of random errors decreases the precision of the assessment, which can be seen in the variability around the estimates, e.g. a large standard deviation or wide confidence interval. Random errors can be minimized by using standardized measurement techniques and by including quality checks. Using a web-based FFQ is one way of reducing random error in dietary assessment since standardization of the

assessment procedure and automatic quality check of answers is easier to apply in a digital format compared to a printed version.

2.2.3 Evaluation of accuracy

The validity and reproducibility of a dietary assessment method is evaluated in a validation study. The validity is evaluated in the comparison with a reference method and the reproducibility is measured with repeated administrations of the same method.

Even if the aim with assessing diet is to capture *true* intake, this can never be obtained with absolute certainty. It would require careful monitoring of subjects over a long time, which poses economical, practical and ethical difficulties, and yet it does not guarantee an observation of true absolute intake. Therefore, a validation study is best described as evaluating *relative* validity where the *test* method is compared to a *reference* method regarded as superior in validity. Ideally, the chosen reference method should have measurement errors independent of the test method so that validity is not overestimated. This can however be difficult to achieve entirely. When evaluating the validity of a FFQ, a common approach is to use multiple food records, 24-hour recalls or biomarkers that cover a specific time period to mirror the time frame of the FFQ. Regarding independency in measurement errors, the FFQ is a retrospective method with predefined food items, frequencies and portion sizes and it relies on memory. The food record on the other hand is prospective and open-ended, has direct assessment of portion sizes and does not rely on memory. The 24-hour recall is however a retrospective method, although it can be open-ended and the reliance on memory is less of an issue. Nevertheless, the FFQ, the food record and the 24-hour recall are all subjected to the issue of social desirability inherent with reporting food intake. Similar to the FFQ, the food record and the 24-hour recall would both have to be linked to a Food Composition Table, which quality also influences the possibility to capture nutrient intake.

Regarding dietary intake, there are unfortunately few *gold standards* to use as reference methods. However, some biomarkers exist. One of them is the doubly labeled water method that measures total energy expenditure[18]. It involves the oral administration of a water dose with known amounts of the stable isotopes of hydrogen (^{2}H) and oxygen (^{18}O) and the subsequent collection of urine samples over a designated time period. The isotopes equilibrate with the body water and ^{2}H is thereafter eliminated via the urine, while ^{18}O is eliminated via both the urine and as carbon dioxide. While the elimination of ^{2}H reflects water turnover, the ^{18}O elimination reflects a measurement of both water turnover and carbon dioxide production. The difference between these eliminations is therefore proportional to carbon dioxide production over the measurement time period. From this, it is possible to calculate the total energy expenditure. This can be used to evaluate the

validity of energy intake assessed with a FFQ and most commonly, the energy intake has been shown to be under-reported.

Ideally, a dietary assessment method should be evaluated in a group representative of the population under study. This is however not always feasible and validation studies therefore sometimes rely on self-selected individuals that might report dietary intake differently from those in the study population. The choice of time frame in a validation study should preferably be made so that the reference method reflects the time frame of the test method. This can however be difficult when the test method assesses past dietary intake as with a FFQ. Then the reference method has to been seen as a proxy for past diet with the assumption that they are reasonably correlated[11].

The reproducibility of a dietary assessment method is made through repeated administration. The time elapse between two administrations of a FFQ is dependent on the time frame of the FFQ. Hence, it is important to try to minimize true within-subject variation affecting the second assessment and thereby the evaluation of reproducibility. The number of food items in a FFQ affects the reproducibility and a short questionnaire can overestimate reproducibility as compared to a long[19].

Several statistical methods are available for evaluating the validity of a FFQ. The more commonly used ones include the initial comparison of mean intakes, cross-classification of quantiles of intake and correlation coefficients. The latter two evaluates the ranking ability of the FFQ. Cross-classification of quantiles also measures the degree of misclassification. To assess the absolute agreement between the FFQ and the reference method, the method by Bland and Altman can be used[20]. With this method, the difference between the FFQ and the reference method is plotted against the average of the two methods. It enables detection of systematic bias as well as potential variance over the intake range. To evaluate the reproducibility of a FFQ, a common approach is to compare mean intakes and to use cross-classification of quantiles as well as analysis of variance using intra-class correlation coefficients (ICC)[21]. ICCs takes into account the within- and between-subject variation and accounts for chance expected agreements.

2.3 NUTRITION AND THE IMMUNE SYSTEM

An adequate nutritional status is vital for a proper functioning of the immune system[1,4,5]. A deficiency of nutrients would hamper the immune response in numerous ways, in particular cell-mediated immunity, cytokine production, phagocyte activity and synthesis of antibodies[5]. The function of immune cells depends to a large extent on cell membrane fluidity, which enables communication between immune cells via membrane-bound receptors. The cell membranes are rich in phospholipids that are sensitive to lipid peroxidation,

which results in deterioration of the fluidity. Therefore, antioxidants play a crucial role in maintaining the structure of immune cells and thereby their function. A deficiency of antioxidants has been seen to suppress immune function and to predispose to infections[5].

Since the immune system is particularly sensitive to oxidative stress[4,5], nutrients with antioxidative properties, such as vitamin C, vitamin E, selenium and zinc, have an essential task in protecting the structure and function of the immune cells[5]. Furthermore, PUFAs have shown to play a role in inflammation[6], which constitutes an important part of the immune function.

2.3.1 Upper respiratory tract infection

The most common illness among humans is acute upper respiratory tract infection (URTI) and adults contract on average 2.5 infections per year[2]. Gender has been found to influence the reaction to respiratory infections[22] and the reported incidence of URTI is higher among women than men[23]. The association with age reveals a decline in incidence with increased age, except for a somewhat higher incidence in the ages 20-39 and an increase at ages above 65, which is explained by immunosenescense[2,24]. URTI includes both the common cold and influenza of which the latter is less frequent. The traditional symptoms of the common cold are nasal discharge, sore throat, cough, sneezing and headache. Influenza symptoms include fever and muscle aches. An URTI usually lasts between 2-14 days, but most recover within a week[25]. Treatment of URTI is not curative but the symptoms can be relieved using medication such as nasal drops, throat drops, cough medication and painkillers.

Most URTI's are caused by viruses. The relative proportion of different viruses are however dependent on different factors such as age and season. The most common causal viruses of URTI are rhinoviruses, which stand for about 30-50% of all infections. Other viruses are coronaviruses, influenza viruses, respiratory syncytial virus and parainfluenza viruses, which are responsible for a smaller proportion of all URTI's. More seldom, URTI can be caused by fungi or bacteria[23,26].

URTI is transmitted through either inhalation of droplets or through contact with droplets with the hands that then touch eyes, nose or mouth[25]. The seasonal pattern of URTI is explained by an increased transmission of viruses in cold weather. Viruses survive longer in cold temperatures and the low humidity also causes dryness of the nasal mucosa, which increases the susceptibility to infections[25]. In addition, the changed social behavior of staying indoors to a larger extent during the winter as well as using public transports more often increases physical contact with others, which further enables transmission of infection.

The seasonal incidence pattern of URTI in Sweden generally stretches from September to April, with a peak in incidence in the very beginning, around December/January and in February[27] (**Figure 2**).

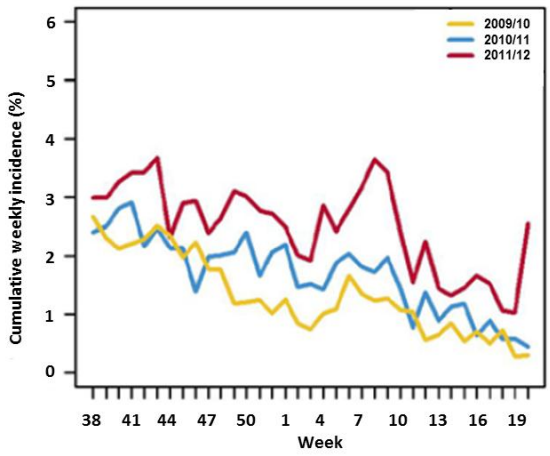

Figure 2. Cumulative weekly incidence of acute respiratory infections for season 2009/2010, 2010/2011 and 2011/2012. Swedish Institute for Communicable Disease Control[27]. Reproduced with permission from the Public Health Agency of Sweden.

During 1995-2006, nearly 52 million annual ambulatory visits occurred in the United States due to acute respiratory infections[28]. Furthermore, the American annual costs for work loss due to URTI are estimated to be more than $20 billion[3]. Despite the individual and societal economic burden of URTI, little is known about how to decrease the susceptibility. However, recent research on dietary intake and susceptibility to infections has provided some support for an association between URTI and certain nutrients[29-34].

2.3.2 Antioxidants and polyunsaturated fatty acids

2.3.2.1 Vitamin C

Vitamin C is a water-soluble antioxidant that can be found in high concentrations in citrus fruits, cabbage, bell peppers, Brussels sprouts, broccoli, cauliflower and berries. In the body, it is found in high amounts in leukocytes where its concentration has been seen to rapidly decrease during an infection[5,35]. It is not fully understood how vitamin C enhances immune function, however since immune cells are particularly sensitive to oxidative stress, the vitamin's antioxidative effect can offer protection to their structure and function[4]. In a recent meta-analysis of 29 randomized controlled trials on vitamin C supplementation and URTI incidence, the risk reduction of URTI in the general

population was quite modest (relative risk: 0.97 (CI 0.94-1.00)), yet both duration and severity of symptoms was significantly decreased[29].

2.3.2.2 Vitamin E

Vitamin E is a fat-soluble antioxidant that can be found in vegetable oils, margarine, meat, poultry, fruit, vegetables, fish and seafood. It protects cell membranes from oxidative damage and also gives resistance to infections by affecting different immune processes[5]. However, mega doses of vitamin E (approximately 300 mg/day) in human and animal cell studies have shown to impair the immune system[36,37]. Randomized controlled trials on vitamin E supplementation and URTI have mainly included elderly (≥60 y) and while one study showed protective effects[30], another study saw no effects but increased severity[31].

2.3.2.3 Selenium

Selenium is a potent antioxidant and a constituent of several proteins, among them the antioxidative enzyme glutathione peroxidase that scavenges reactive oxygen species (ROS) and thereby protects cells in the immune system[5]. The Swedish soil is low in selenium why the main dietary sources are meat, poultry, fish, seafood, milk and egg. Animal and human cells studies have showed selenium status to affect influenza susceptibility[38,39]. The mineral itself also enhances the resistance to infection through modulation of the Th1/Th2 response[5]. Nevertheless, an excessive intake of selenium has been shown to harm the immune system[40].

2.3.2.4 Zinc

Zinc is an antioxidant which main dietary sources are meat, milk and dairy products as well as wholegrain cereals. It is a co-factor for the antioxidative enzymes superoxide dismutases and also affects maturation and differentiation of T cells in the immune system[5]. In-vitro studies have showed that zinc can inhibit growth of eight of nine strains of rhinoviruses[41]. Nevertheless, high zinc levels may act pro-oxidative[35] and routine supplementation has shown adverse effects on the immune system[42]. Zinc supplementation and URTI has been studied in several trials, whereof two of them showed a decreased incidence among children[32,33] and 14 studies a reduction in duration[34].

2.3.2.6 Polyunsaturated fatty acids

PUFAs, i.e. omega-6 and omega-3, are found in margarines and vegetable oils. Omega-3 fatty acids are also found in fatty fish, such as salmon and herring. Among the omega-6 fatty acids, arachidonic acid (AA) is of special interest in

nutritional immunology. Both AA and omega-3 are abundant in immune cells and the content can be altered through dietary intake[6]. AA can be transformed into eicosanoids, which act in inflammatory processes[43,44], whereas omega-3 can form anti-inflammatory resolvins[6].

Even if randomized controlled trials previously have investigated the effect of vitamin C, vitamin E and zinc on URTI, there might be different biological effects of the nutrients depending on if they originate from food or supplements. We therefore believe that more studies on dietary intake of these nutrients and URTI are needed. To our knowledge, there are no previous cohort studies or trials on selenium intake and URTI. Moreover, although PUFAs have been shown to affect the immune function, to our knowledge, no studies have so far investigated the association between PUFA intake and URTI.

2.3.3 The Nordic Nutrition Recommendations (NNR)

Since 1980, the Nordic countries (Sweden, Norway, Denmark, Finland, and Iceland) have together published joint guidelines for dietary composition and nutrient intake, the *Nordic Nutrition Recommendations* (NNR)[45,46]. The NNR include recommendations on total energy intake, macronutrients as a percentage of total energy intake, intake of vitamins, minerals, fiber and salt, as well as recommendations on physical activity. The recommendations are based on the current nutritional situation in the Nordic countries and available scientific knowledge. The main purpose is to provide guidelines to promote overall good health and to reduce the risk of diet-associated diseases in the general healthy population.

Dietary guidelines have previously been evaluated for chronic diseases such as cardiovascular disease and cancer[47-52] . However, to our knowledge, no studies have so far investigated the adherence to nutrition recommendations and risk of URTI. Since 2004, recommendations on physical activity are included in the NNR. The effect of physical activity on immune function has been investigated before and physically active individuals were found to have a lower risk of contracting URTI[7,9,10,53-59].

3 AIMS

The overall aim of the thesis is to evaluate the validity of two newly developed web- and meal-based FFQs, Meal-Q and MiniMeal-Q, and to study the potential association between dietary intake and susceptibility to upper respiratory tract infection.

For each study, the specific aims are:

Paper I: to evaluate the validity of Meal-Q and MiniMeal-Q regarding intake of energy and macronutrients as well as to evaluate the reproducibility of Meal-Q.

Paper II: to evaluate the validity of Meal-Q and MiniMeal-Q regarding intake of micronutrients and fiber as well as to evaluate the reproducibility of Meal-Q.

Paper III: to study the adherence to the Nordic Nutrition Recommendations and susceptibility to upper respiratory tract infection.

Paper IV: to study the association between antioxidants and polyunsaturated fatty acids and susceptibility to upper respiratory tract infection.

4 METHODS

4.1 VALIDATION STUDY – VALMA

4.1.1 Background

Meal-Q was developed to be used as a web-based FFQ in a large prospective population-based cohort study, *LifeGene*[60]. The *LifeGene* study is a national collaboration project between several universities in Sweden and investigates the interplay between heredity, environment and lifestyle factors on different health outcomes. The data collection is entirely web-based and dietary intake is one of many investigated exposures assessed in a large web-questionnaire. It was therefore important to design a FFQ with short answering time, yet still comprehensive enough to assess habitual dietary intake.

The development of Meal-Q was based on a population-based study among 700 randomly selected Swedish individuals in which data on food products and beverages consumed for breakfast, lunch, dinner and snack meals was collected through face-to-face interviews or 24-hour telephone recalls (E Möller and S Christensen, personal written communication, August 2008). The information obtained from this study guided the design of a meal- and web-based FFQ called MaxMeal-Q. After a performance test of MaxMeal-Q in a randomly selected group of individuals (N=216), we omitted less commonly consumed food items and dishes and re-named it Meal-Q. Subsequently, Meal-Q was included in the Validation of Methods Assessing diet and physical activity (VALMA) study. After the completion of the validation study, *LifeGene* decided to use Meal-Q under the condition that the answering time could be reduced. We therefore developed the shorter version MiniMeal-Q by omitting food items with low intake frequency as well as low contribution to total energy and nutrient intake. However, food items that represented important food sources of certain nutrient intakes were kept, e.g. black pudding, which contributed to iron intake. After a time test, the *LifeGene* study decided to use MiniMeal-Q.

4.1.2 Study design and population

The VALMA study was conducted to evaluate the validity and reproducibility of Meal-Q. After the study was completed, we evaluated the shorter version MiniMeal-Q by using truncated Meal-Q data. Besides Meal-Q, the VALMA study included a classic food group-based FFQ (Classic FFQ) previously used in many studies[61-63] and a web-based questionnaire on physical activity, Active-Q[64]. However, the description of the validity of the Classic FFQ and Active-Q is beyond the scope of this thesis. As reference methods to evaluate Meal-Q and MiniMeal-Q, we used a 7-day weighed food record (WFR) for evaluation of energy and nutrient

intake and the doubly labeled water (DLW) method[18] for measurement of total energy expenditure. All participants filled out the WFR, while only a subgroup drank DLW. The study was limited to a total of three weeks since the enrollment of the LifeGene study was to take place soon after the validation study.

In the spring of 2009, we recruited a total of 180 healthy volunteer men and women aged 20-63 through public announcement in Stockholm County, Sweden. The announcements were put up in the city, the suburbs and at two universities. To be eligible, participants were required to have Internet access and an e-mail address and were not supposed to be on a weight-loss diet, nor being pregnant or having given birth during the last ten months. At an introductory meeting, all subjects were informed about the study and gave their written informed consent. The Research Ethics committee at Karolinska Institutet approved the study.

The participants were divided into three age- and gender-balanced groups: group 1 (n=87), group 2 (n=53), and group 3 (n=40) and each group followed a 3-week study scheme as shown in **Figure 3**. All participants filled out Meal-Q and the WFR on the web at their own choice of location. Groups 3 were also given DLW. For validity evaluation, data from the first administered Meal-Q assessment and the WFR from all groups were compared. For reproducibility evaluation, group 2 and 3 filled out a second Meal-Q after three weeks. To evaluate the validity of MiniMeal-Q, the data from the first Meal-Q assessment was truncated and compared with the WFR. Answering time was automatically recorded, and directly after completion of the first Meal-Q, a short Web survey followed to evaluate its user-friendliness.

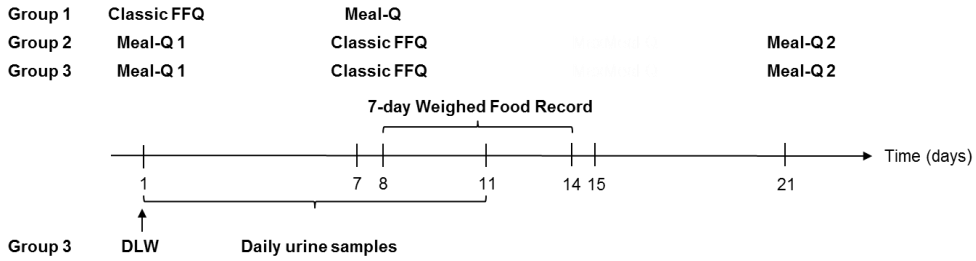

Figure 3. Study scheme of VALMA.

4.1.3 Dietary assessment

Meal-Q

Meal-Q is an interactive web-based FFQ that includes 102-174 food items depending on the number of follow-up questions. The interactivity implies follow-up questions for high consumers of certain food items and dishes. In the VALMA

study, it asked about dietary intake during the past few months. Meal-Q assesses intake of (1) food items, dishes, and beverages, (2) energy and nutrients, including alcohol, (3) supplements, (4) meal patterns, and (5) eating behavior, such as restaurant visits, intake of fast food, light products, probiotics, and the use of cooking fat and salt. Respondents choose among predefined food items and intake frequencies ranging from 1-3 times a month to 5+ times a day. For each of the following food groups, five photos of portion sizes are included: (1) rice, potatoes, and pasta, (2) meat, chicken, fish, and vegetarian substitutes, and (3) vegetables (raw or cooked). The photos are used to calculate portion sizes for cooked dishes and vegetables, while standard portion sizes are used for other food items. The standard portion sizes are derived from the National Food Agency, the Swedish Consumer Agency, measured portion sizes developed by the research group and standard portion sizes used in other FFQs at Karolinska Institutet.

MiniMeal-Q

MiniMeal-Q includes 75-126 food items (approximately 30% fewer items than Meal-Q) and also covers most of the questions on meal patterns and eating behavior as included in Meal-Q. After the validation study was completed, MiniMeal-Q was sent out to 79 volunteer VALMA participants to assess answering time and user-friendliness.

7-day Weighed Food Record on the web

At the introductory meeting, all participants were given oral instructions on how to fill out the web-based WFR (Energibalans.nu). They were also given a kitchen scale and a handbook with instructions on how to weigh all their consumed food products and beverages at the highest detail level possible. For example, a dish was encouraged to be reported by its individually weighed food items. The WFR included a database with over 2000 food items to choose from. All records were checked for completeness and reasonableness by the data collectors.

Nutrient Database

The intake of food items and dishes from Meal-Q, MiniMeal-Q, and the WFR were converted into energy (kJ/day), macro- and micronutrient (g/day, mg/day or µg/day) intake using the national Food Composition Table provided by the Swedish National Food Agency[65]. The conversion for the questionnaires was done by programs developed and validated specifically for the VALMA study (MealCalc, MiniMealCalc), whereas the conversion of the WFR was made within the web-based WFR program. The nutrients from dietary supplement intake were not included in the analyses.

4.1.4 Doubly labeled water method

Total energy expenditure was assessed in group 3 (n=40) using the DLW method[18] over 11 consecutive days (Figure 3). The details of this procedure have been described previously[64]. Briefly, on day 1 at the study site, each participant provided a 5-ml urine sample before receiving an oral DLW dose calculated according to body weight[66]. Subsequently, daily spot urine samples were collected for a total of 9 days. Participants were instructed to collect urine samples at a similar time each day (but not the first void of the day). All samples were kept refrigerated. On day 11, the 9 urine samples were returned to the study site and the eleventh urine sample was collected. All samples were shipped to the Medical Research Council, Human Nutrition Research, Cambridge, United Kingdom, for isotopic analysis, which has been previously described in detail[67]. Enrichments of 2H/1H and 18O/16O in urine samples were determined by mass spectrometry. Following conversion to the universally accepted Vienna Standard Mean Ocean Water (VSMOW) / Standard Light Arctic Precipitation (SLAP) scale, total energy expenditure was calculated by using standard equations[68-70]. CO_2 production (mole/day) was estimated using Schoeller et al's correction for fractionation[69] and a respiratory quotient of 0.85. The respiratory quotient is based on omnivores with 30-35% energy contribution from fat and suitable to the VALMA population. The results of the CO_2 production were used to calculate the total energy expenditure of each participant by using the modified Weir equation[71].

4.1.5 Assessment of physical activity for identification of energy under-reporters

The WFR program included a 7-day pedometer-based physical activity record provided for all participants. The participants were asked to report their total number of daily steps as well as other activities not captured by pedometers, such as bicycling or swimming. The information was thereafter used to calculate the physical activity level (PAL). Individual PAL values were also obtained from measurements of total energy expenditure by DLW in group 3. By using the Goldberg cut-off method[72], the PAL values derived from the pedometers and the DLW measurements were used to identify potential under-reporters of energy intake in the WFR in order to exclude them from the validity analyses of Meal-Q and MiniMeal-Q.

4.1.6 Statistical methods

Median and interquartile range (IQR) of crude energy, micro- macronutrient intake was calculated and compared between Meal-Q, MiniMeal-Q, and the WFR. Energy intake from the questionnaires was also compared to total energy expenditure from DLW. Wilcoxon signed rank tests were used to determine differences between the methods.

The median (IQR) answering time in minutes of each questionnaire was calculated and the user-friendliness was evaluated from the web survey. Further, linear regression was used to obtain the between-person variance captured in the truncated MiniMeal-Q as compared to Meal-Q. The Goldberg cut-off method[72] was used to identify energy under-reporters and was calculated using the energy intake from the WFR together with the obtained PAL values from the physical activity record and the DLW data.

For validity and reproducibility analyses, micro- and macronutrients were adjusted for total energy intake using the residual method[73] and non-normally distributed variables were transformed using the square, square root, or log transformation. The absolute agreement and potential difference in bias over the intake range was evaluated with the Bland-Altman method by plotting the differences between questionnaires and WFR or DLW against the average of the two methods[20]. The degree of variation was represented by the limits of agreement, i.e. ±2 SD of the mean difference. To test the ranking agreement and magnitude of misclassification of the questionnaires in comparison to the WFR and DLW we used quartile cross-classifications and calculated proportions of subjects classified into the same, adjacent and extreme quartiles of energy-adjusted intakes. Further, Pearson and Spearman correlation coefficients were used to measure ranking ability and the degree of linear relationship between the questionnaires and the WFR and DLW. Pearson correlations were used for macronutrients since they were normally distributed after energy adjustment and transformation, whereas Spearman correlations were used for micronutrients and fiber since they were non-normally distributed. By using the formulas of Beaton et al[74] and Liu et al[75] we calculated de-attenuated correlations corrected for within-person variation in the WFR, and 95% confidence intervals (CI) were produced using the method of Willett and Rosner[76]. Confidence intervals for correlations with DLW were obtained using the bootstrap method[77].

The reproducibility of Meal-Q was evaluated by comparing crude median energy, micro- and macronutrient intake between the first and second Meal-Q and by cross-classification of energy and energy-adjusted quartiles of micro- and macronutrient intake. Furthermore, we computed intraclass correlation coefficients (ICCs)[21] using 1-way ANOVA with random effects.

4.2 PROSPECTIVE COHORT STUDIES – LIME AND SWEDE-I

4.2.1 Study design and populations

The **LIME** study is a population-based cohort study on lifestyle factors and URTI. A total of 5,000 men and women aged 20-60 years and residing from a northern

Swedish middle-sized county (Umeå) were randomly selected from the Swedish Total Population Registry. Invitations to participate were sent out in January 2004 via regular paper mail with information on how to access a Web-based questionnaire about lifestyle factors including dietary intake. The questionnaire was completed by 1,805 individuals and after exclusions, 1,509 were eligible for continued participation. Participants were excluded if they had an URTI at baseline (n=236), lacked an e-mail address (n=17) or chose not to disclose it (n=20). Additional exclusions of 23 participants were made for whom the e-mails were filtered as junk mail. During a follow-up period of 15 weeks, from February to May, five follow-up questionnaires were sent out with questions on URTI incidence during the three preceding weeks. Non-responders got reminders 1.5 weeks after each follow-up. The response rate for the follow-up questionnaires was 83-84% and in total, 1,111 participants completed all five follow-ups. A flowchart of the data collection can be seen in **Figure 4**. The Ethics Committee at Karolinska Institutet approved the study.

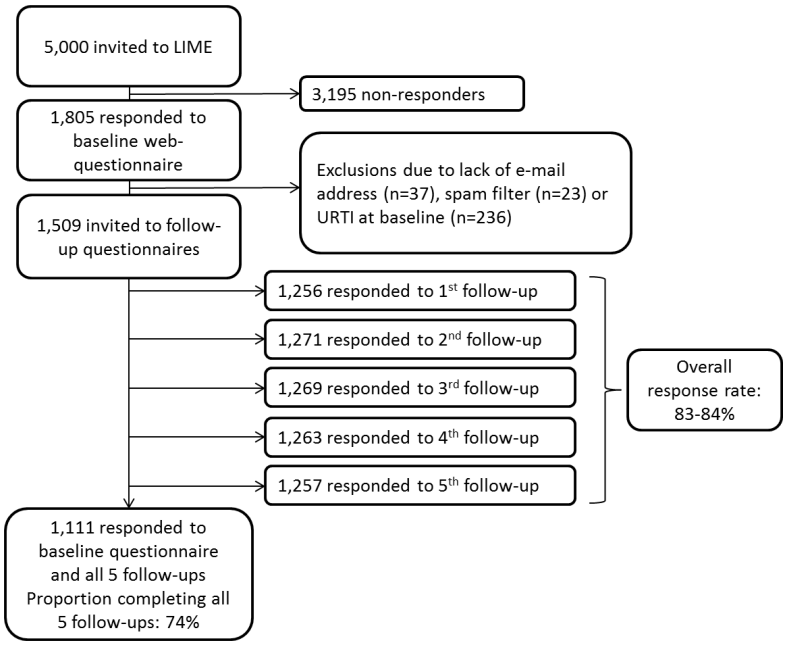

Figure 4. Flowchart of the data collection in the LIME study.

The **SWEDE-I** study (Studies of Work Environment and Disease Epidemiology-Infections) is a prospective cohort study on work-related risk factors for respiratory and gastrointestinal viral infections. In the summer of 2011, a total of 14,008 individuals from Eskilstuna municipality in Sweden were invited to participate. The selection of individuals was made by Statistics Sweden by cross linkage of the Labor Market Register and the Total Population Registry to render an age- and gender stratified random sample. A total of 2,450 individuals agreed

to participate, whereof 188 did not meet the study criteria for participation (working full- or part-time) and 25 were excluded due to administrative errors. In total, 2,237 women and men aged 25-64 years were enrolled in the study starting on August 22nd with just over nine months of follow-up until May 31st 2012. The participants were followed passively and were instructed to report URTI events as they occurred. The mean (SD) follow-up time was 32 (15) weeks and complete dietary data was collected for 1,533 subjects. A study scheme of the SWEDE-I study can be seen in **Figure 5**. The Ethics Committee at Karolinska Institutet approved the study.

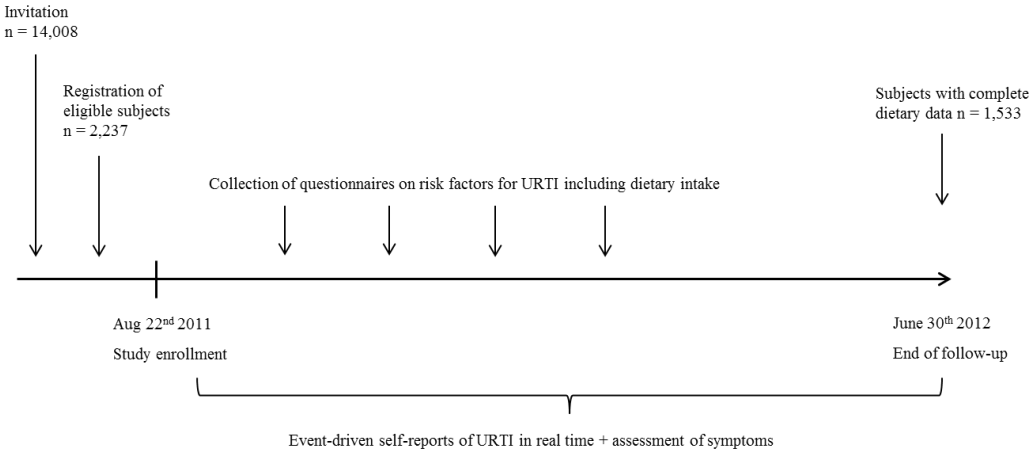

Figure 5. Study scheme of the SWEDE-I study.

4.2.2 Assessment of exposure

The LIME study

Dietary intake was assessed at baseline by a food group-based 96-item validated FFQ[78], which was originally developed as a paper-questionnaire, but made web-based for this study. The FFQ assessed habitual dietary intake including vitamin and mineral supplements. Data from the questionnaire was linked to the Food Composition Table of the Swedish National Food Agency[79] to calculate the daily mean intake of nutrients using the program NUDES. In a validation study comparing the FFQ to a 7-day food diary, the Spearman correlation coefficients range from 0.38 (iron) to 0.81 (vitamin C) for micronutrients and were the following for macronutrients: 0.44 (protein), 0.73 (carbohydrates), 0.71 (fiber), 0.70 (total fat), 0.75 (saturated fat), 0.66 (monounsaturated fat), 0.49 (polyunsaturated fat) and 0.81 (alcohol).

Total physical activity and inactivity was assessed at baseline using a validated questionnaire[80]. Participants were asked to report the amount of time (hours and

minutes) spent on nine activity levels (including sleep) on a typical day and night, adding up to 24 hours. Each activity was explained by examples of activities corresponding to a MET-value (metabolic equivalent task, multiples of resting metabolic rate)[81,82]. The average MET-hours expended during a 24-hour period were estimated by multiplying the time spent at each activity level with its corresponding MET-level. The MET-values for the nine different activity levels ranged from 0.9 to 8.0 where 1 MET corresponds to sitting in a relaxed position, 3-5 METs equals walking at a moderate to brisk pace, housework or playing golf, and 6 METs include mowing the lawn with a hand mower or jogging at a slow pace[81,82]. The hours and minutes spent at the different activity levels were automatically summed in the web questionnaire and participants got reminded if their total sum did not add up to 24 hours. From the average MET-hours for each participant, we estimated the amount of time (min/day) spent at moderate (5 METs) or vigorous (>6 METs) activity. The NNR 2004 recommends at least 30 minutes and preferably more than 60 minutes of moderate and/or vigorous physical activity per day in addition to inactive living[45].

The NNR 2004 includes recommendations on macronutrients as percentage of total energy intake as well as recommendations on absolute daily intake of vitamins, minerals, fiber, salt and on physical activity [45]. The recommendations were divided into six groups; intake of 1) macronutrients (protein, carbohydrates and fat), 2) micronutrients (vitamins and minerals), 3) sodium, 4) alcohol, 5) fiber, and 6) physical activity. For each group, every individual recommendation was graded on a continuous scale of 0-1. Intakes within the recommendations were given 1 point and 0 point was given to intakes below a defined lowest value and/or above a highest value (for nutrients, the median of the ten lowest and/or ten highest intakes among the study population was used, and for physical activity, <30 min/day was used as the lower value). A relative score of 0-1 points was given for intakes or activity levels in between the recommended level and the above defined lowest and highest values according to the following calculation (where Y is the new adherence variable and X is the intake or activity level):

For lower limits, Y varies from 0 to 1:
Y = (X-lowest value)/(lower NNR cut-point-lowest value)

For upper limits, Y varies from 1 to 0:
Y = 1-(X-higher NNR cut-point)/(highest value–higher NNR cut-point)

The individual scores were summed up and divided by the number of individual recommendations included in the group to get an overall score for the groups of recommendations (e.g. fat, carbohydrates, vitamins and minerals). In this way, each group was given equal weight in the final adherence score. Sodium, alcohol, fiber and physical activity were kept as individual recommendations. A total score of 0-6 points was thereafter made by summing up all scores from the six

recommendation groups. The score was further divided into three arbitrary cut-points; <4.5 points for low adherence (range 0.17-4.49, median 4.10), 4.5-5.5 (median 4.99) points for medium adherence and >5.5 points for high adherence (range 5.50-5.83, median 5.60). The low adherence group was used as reference category in the statistical analysis.

To evaluate the effect of the scoring *per se*, three additional scoring models were tested. The first model gave more weight to the score for vitamins and minerals. The second model gave more weight to the score for vitamins, minerals and individual macronutrients. Lastly, the third model only included recommendations that were open-ended (e.g. ≤10% of energy from saturated fat) and excluded those that were not open-ended (intake of total fat, monounsaturated fat, polyunsaturated fat, protein and carbohydrates). In that way, measurement error in the FFQ would have less influence on the results.

The SWEDE-I study

Dietary and supplement intake was assessed by the means of a validated web- and meal-based FFQ, MiniMeal-Q[83,84]. The questionnaire includes 75-126 food items and is interactive with follow-up questions, which adapts it to the respondents' dietary habits. It asks about habitual dietary intake and assesses intake of food items, dishes, beverages, energy, nutrients and the use of dietary supplements. Spearman correlation coefficients between MiniMeal-Q and a 7-day weighed food record were r=0.54 for vitamin C, r=0.48 for vitamin E, r=0.44 for selenium and r=0.35 for zinc[83]. The Pearson correlation coefficient for PUFAs was r=0.40[84]. MiniMeal-Q was completed by 1,533 subjects, whereof 926 women and 607 men. Information on dietary intake was linked to the Food Composition Table of the Swedish National Food Agency[65] and calculation of daily mean intake of energy and nutrients was thereafter made using a nutrient calculation program developed and validated by the research group (MiniMealCalc).

Energy-adjusted nutrient intake from food was categorized separately for women and men into three levels (low, medium and high intake) based on the distribution of intake. The lowest level was used as reference category. For women, the categories for vitamin C were: <45, 45-110, >110 (mg/d); for vitamin E: <6.5, 6.5-9.5, >9.5 (mg/d); for selenium: <25, 25-45, >45 (µg/d); for zinc: <7.5, 7.5-11, >11 (mg/d); for omega-6: <0.45, 0.45-1.25, >1.25 (g/d); for AA: <40, 40-85, >85 (mg/d); for omega-3: <0.13, 0.13-0.5, >0.5 (g/d); for eicosapentaenoic acid (EPA): <30, 30-100, >100 (mg/d); and for docosahexaenoic acid (DHA): <0.1, 0.1-0.32, >0.32 (g/d). For men, the categories for vitamin C were: <40, 40-100, >100 (mg/d); for vitamin E: <6, 6-8.5, >8.5 (mg/d); for selenium: <25, 25-40, >40 (µg/d); for zinc: <7, 7-10, >10 (mg/d); for omega-6: <0.4, 0.4-1.2, >1.2 (g/d); for AA: <40, 40-80, >80 (mg/d); for omega-3: <0.13, 0.13-0.45, >0.45 (g/d); for EPA: <25, 25-100, >100 (mg/d); and for DHA: <0.1, 0.1-0.35, >0.35 (g/d).

Dietary supplement use of vitamin C and/or multivitamin/minerals as well as of omega-3 fatty acids was divided into two categories: daily/weekly or monthly/in period/no use with the latter used as reference category. Supplement use of vitamin E, selenium and zinc was rare and therefore not evaluated.

Potential confounding factors

There are potentially numerous confounding factors between dietary intake and URTI. For example, the incidence of URTI has been shown to vary with age, being the highest in the early years of life and then decrease until age 65 when the immunosenescense starts deteriorate the immune function[23,85]. Furthermore, the response to respiratory tract infections has been found to be influenced by gender[22] and adult men experience URTI less frequently than women[23]. A cohort study among children showed that obese children were more inclined to have a serious cold or influenza than normal weight children[86]. Obese adults have also been found to experience a higher morbidity and mortality from the H1N1 influenza according the Centers for Disease Control and Prevention[87]. Season affects the transmission of viral infections in several ways. The cold weather enables viruses to survive longer than in warmer temperatures and the low humidity increases the susceptibility to infections through increased dryness of the nasal mucosa. In addition, people stay indoors to a larger extent during the winter, which increases physical contact with others and further enables virus transmission. Smoking has been shown to worsen and prolong URTI symptoms[88] and alcohol intake has been found to suppress the immune response and lead to increased risk of infections[89]. Both stress and physical activity have been found to affect the susceptibility to URTI[7-10,90,91]. Moreover, since pollen allergy generates similar symptoms as URTI, it is important to adjust for pollen allergy to disentangle their symptoms. Chronic illness and medications that affects the immune function are also important factors to control for.

Dietary intake as well as nutrient metabolism is likely to be influenced by the above mentioned factors, whereof smoking and alcohol has been studied in more detail[92,93]. Furthermore, other nutrients could be potential confounders, e.g. beta-carotene, folate and iron, which all affect the immune system[5] and perhaps also influence the intake and/or metabolism of the studied nutrients.

In the **LIME** study, potential confounding for the association between dietary intake and URTI was evaluated for age, sex, children, weight, energy intake, education, smoking, snuff use, medication, influenza vaccination history, self-reported weakened immune system, pollen allergy, asthma, stress, poor sleep, use of public transport, contact with large crowds at leisure time and season. In the **SWEDE-I** study, the following variables were evaluated for potential confounding between dietary intake and URTI: age, sex, BMI, education, smoking, physical

activity, small children, flu vaccination, chronic illness, sleep quality and duration, weakened immune system, pulmonary disease, alcohol, beta-carotene, folate, iron and supplement intake of vitamin C, D, E and A, antioxidant, multivitamin/mineral, selenium, zinc, omega-3, folate and iron. For supplement intake and its association with URTI, we evaluated dietary intake of PUFAs, vitamin C and E, selenium, zinc, beta-carotene, folate and iron as well as supplement intake of antioxidants, vitamin E, selenium, zinc, vitamin A, vitamin D, folate and iron.

4.2.3 Ascertainment of URTI

In the **LIME** study, URTI was self-reported at baseline and in five web-based follow-up questionnaires sent out to the participants between February and May. The participants were asked whether they currently had an URTI or had had an URTI event during the three preceding weeks. An URTI event was recorded if answering "yes" to this question. The participants were instructed not to count an URTI event twice, even if it crossed over two follow-up periods. Those who reported an infection got follow-up questions on seven symptoms: sore throat, cough, runny nose, headache, malaise, fever and unspecified symptoms. Information on influenza vaccination history was collected at baseline and updated at all five follow-ups. Since pollen allergy can mimic URTI symptoms, information on pollen allergy was collected in the follow-up questionnaires in April and May (i.e. the season for pollen where the study was conducted).

In the **SWEDE-I** study, URTI was self-reported throughout the study on an event-driven basis. The subjects were instructed to report as soon as they believed that they had a cold. Reporting was made through interactive voice response service (IVRS) via regular telephone or through a secure web-based form. Information on symptoms was collected for sore throat, coughing, runny nose, headache, fever and body ache. An URTI event was defined as the presence of runny nose, sore throat or coughing or the presence of either two or all three symptoms. An URTI event with onset date at least four days after a previous URTI was considered a new event. Reminders in the form of newsletters were sent out on six occasions during the data collection.

4.2.4 Statistical methods

The Poisson regression model is based on the Poisson distribution and uses maximum-likelihood estimation to model count data. The Poisson distribution describes the probability of a given number of events (counts) that occur during a defined time interval with the assumptions that the rate of the events is constant and that every event is independent of other occurring events. Each event should also be clearly defined and not counted more than once.

The Cox proportional hazards regression model is a semi-parametric model used for survival analysis. The hazard function consists of two parts, the baseline hazard function of time that is allowed to vary freely without any parametric constraints, and a parametric function of the covariates.

The LIME study

The Poisson regression model was used to estimate incidence rate ratios (IRR) with 95% confidence intervals (CI) and to control for potential confounding factors. Participants free of URTI contributed three weeks of person-time for each 3-week follow-up period. Since the exact time point for an URTI onset or its duration was unknown, a participant with a reported URTI contributed 1.5 weeks of person-time for the corresponding 3-week follow-up period. To evaluate whether each URTI event was independent of other URTI events, a generalized estimating equations (GEE) model was used to account for repeated events. A negative binomial model that assumes every event to be independent was also tested. The results from the GEE model and the negative binomial model were similar to the Poisson model in all analyses. Potential confounding factors were identified by eliminating one factor at a time from the full model. A variable was considered a confounding factor if it changed the point estimate with more than 10%.

Cubic spline regression models[94] were used to identify the shape of the relationship between NNR adherence and URTI. The models were fit with six knots at minimum, maximum, and at adherence scores 1.0, 2.0, 3.0 and 4.0. A graph of the rate was presented at the reference value for all other covariates predicted from the model.

The goodness of fit for the Poisson regression model can be tested by studying deviance or Pearson's chi-square statistic divided by degrees of freedom (DF) to evaluate the presence of either over- or underdispersion. Overdispersion occurs when there is excess variation around fitted values than predicted by the model, whereas underdispersion occurs when there is lower variation than expected. The results from this evaluation showed little evidence for over- or underdispersion in the data (deviance/DF=0.82 and chi-square/DF=1.62, both compared to a chi-square distribution with 1 DF).

The SWEDE-I study

The Cox proportional hazards regression model was used to calculate incidence rate ratios (IRR) with 95% confidence intervals (CI) using the lowest intake group as reference category. We let the Cox model allow for multiple URTI events per subjects. By including an interaction term between sex and the exposure, estimated effects of exposure were obtained separately for women

and men. Potential dependency between URTI events was controlled for using the robust sandwich estimator for standard errors.

Confounding factors were evaluated by forward addition to each model and a covariate was kept if the point estimate changed by ≥10%. Effect measure modification was evaluated by including an interaction term between the main exposure and the covariate. Moreover, interaction with time was evaluated by including a time-varying covariate in the model. Sensitivity analyses were done for pollen allergy and time of dietary assessment. Since pollen allergy can mimic URTI symptoms, all models were run without subjects reported having pollen allergy to evaluate if the point estimate changed. The date of filling out MiniMeal-Q was also evaluated in order to investigate if it affected the reported intake and thereby the point estimate.

Restricted cubic spline models[94,95] with three evenly distributed knots were used to graphically evaluate a potential dose-response relationship between dietary intake and URTI while controlling for confounding factors.

5 RESULTS

5.1 VALIDATION STUDY – VALMA

Results are presented jointly for **Paper I** and **Paper II**.

Paper I - *Two new meal- and web-based interactive food frequency questionnaires: validation of energy and macronutrient intake*

Paper II - *Relative validity of micronutrient and fiber intake assessed with two new interactive meal- and web-based food frequency questionnaires*

5.1.1 Subject characteristics and descriptive results

From the initial 180 subjects, one was excluded due to drop-out (group 1) and two due to illness (group 2 and 3). In addition, we excluded 14 subjects (four in group 1, six in group 2 and four in group 3) identified with the Goldberg cut-off method as energy under-reporters in the WFR. In total, 163 subjects remained for validity evaluation; group 1: n=82; group 2: n=46; and group 3: n=35. One subject that had implausibly high intake of beta-carotene (>30 000 µg/day) and three other subjects that had implausibly high intakes of sodium (>9000 mg/day) in the WFR were excluded in each respective nutrient analysis. For reproducibility evaluation in group 2 and 3, four subjects had missing values in the second administered Meal-Q, leaving 87 subjects in the analysis. We found no significant differences between included and excluded subjects in terms of age, BMI, education, nutrition background or tobacco use (P = 0.16-1.00).

The study population consisted of mainly women (79%) with a mean (SD) age of 33 (12) years and a mean (SD) BMI of 23 (3.6). A majority of the subjects were highly educated or students. A third was working full time and about as many had training in nutrition. Few subjects used tobacco. There were no differences between study groups or sexes regarding age, BMI, education, nutrition background, smoking or multivitamin/mineral supplement use (P = 0.05-1.00). However, more men than women used Swedish snuff (P =0 .001).

Meal-Q and MiniMeal-Q had a median (IQR) answering time of 17 (11) and 7 (4) minutes, respectively. A total of 92% perceived Meal-Q as easy to fill out, 91% thought the questions were relevant and 93% reported that food items and dishes were presented in a logical order. For MiniMeal-Q, the figures were 95%, 88% and 91%, respectively. The overall mean grade of Meal-Q and MiniMeal-Q's user-friendliness was 4.2 on a 5-point scale where 5 was the best grade. The between-person variance captured by MiniMeal-Q as compared to Meal-Q ranged from

96% to 99% for energy and macronutrients and from 70% to 100% for micronutrients and fiber.

5.1.2 Validity

The median crude daily intake of energy, macro- and micronutrients as assessed with the WFR, Meal-Q and MiniMeal-Q as well as the total energy expenditure measured with the DLW method is shown in **Table 1** and **2**. The median (IQR) intake of energy and most macro- and micronutrients was assessed higher with the WFR than with the questionnaires. The total energy expenditure measured with the DLW method was also higher than the energy intake assessed by both questionnaires and the WFR. There was no statistically significant difference in assessments between Meal-Q and MiniMeal-Q.

Table 1. Median (IQR) daily crude intake of energy and macronutrients assessed with the WFR, Meal-Q and MiniMeal-Q (n=163) and total energy expenditure measured with DLW in group 3 (n=39).

	WFR	Meal-Q[a]		MiniMeal-Q[a]	
	Median (IQR)	**Median (IQR)**	**% of WFR**	**Median (IQR)**	**% of WFR**
All groups (n=163)					
Energy (kJ)	9183 (2340)	7667 (3723)	83	7017 (3632)	76
Protein (g)	85 (37)	79 (40)	93	70 (34)	82
Carbohydrates (g)	243 (97)	211 (132)	87	190 (124)	78
Total fat (g)	86 (37)	65 (34)	76	62 (35)	72
Saturated fat (g)	33 (18)	22 (14)	67	20 (13)	61
Monounsaturated fat (g)	31 (16)	23 (13)	74	22 (11)	71
Polyunsaturated fat (g)	14 (8)	13 (8)	93	12 (9)	86
Alcohol (g)	6 (15)	5 (8)	85	5 (8)	83
	DLW	**Meal-Q**	**% of DLW**	**MiniMeal-Q**	**% of DLW**
Group 3 (n=39)					
Energy (kJ), median (IQR)	11423 (2777)	7954 (2736)[b]	70	7358 (2718)[b]	64

[a] Assessments with Meal-Q and MiniMeal-Q were statistically significantly different from the WFR (P =0 .01), except for polyunsaturated fat assessed with Meal-Q (P =0 .28). Intakes assessed with Meal-Q and MiniMeal-Q were not statistically significantly different from each other (P <0.001). (Wilcoxon's signed-rank test).

[b] Energy intake was statistically significantly different from energy expenditure (P < 0.001).
(Wilcoxon's signed-rank test)

Table 2. Median (IQR) daily crude intake of micronutrients and fiber[a] assessed with the WFR, Meal-Q and MiniMeal-Q (n = 163).

	WFR		Meal-Q		MiniMeal-Q	
	Median	**(IQR)**	**Median**	**(IQR)**	**Median**	**(IQR)**
Beta-carotene (µg)	2632[b]	(2539)[b]	3372	(2905)	3254	(3079)
Thiamine (mg)	1.5	(0.5)	1.4	(0.8)	1.3	(0.8)
Riboflavin (mg)	1.9	(0.7)	1.7	(0.9)	1.5	(0.8)
Niacin (mg)	36	(14)	15	(9)	14	(8)
Vitamin B6 (mg)	2.3	(1.0)	1.8	(1.0)	1.7	(0.9)
Folate (µg)	334	(167)	315	(210)	289	(193)
Vitamin B12 (µg)	5.7	(3.7)	3.8	(2.4)	3.5	(2.3)
Vitamin C (mg)	121	(92)	101	(82)	94	(74)
Vitamin D (µg)	5.6	(4.0)	4.7	(3.3)	4.4	(3.1)
Vitamin E (mg)	11	(5)	10	(5)	9	(5)
Calcium (mg)	1052	(381)	897	(583)	828	(512)
Iron (mg)	13	(6)	13	(7)	11	(6)
Magnesium (mg)	413	(177)	397	(242)	358	(207)
Phosphorus (mg)	1570	(514)	1433	(731)	1305	(677)
Potassium (mg)	3437	(1332)	3130	(1600)	2837	(1477)
Selenium (µg)	45	(21)	44	(24)	36	(22)
Sodium (mg)	3194[c]	(1212)[c]	2448	(1118)	2158	(1015)
Zinc (mg)	12	(4)	11	(5)	10	(5)
Fiber (g)	25	(15)	26	(20)	23	(18)

[a]Most nutrients assessed with the WFR were higher than the intakes assessed with Meal-Q and MiniMeal-Q (P = 0.00-0.03). Exceptions were beta-carotene that was assessed higher with Meal-Q (P = 0.03), but did not differ between the WFR and MiniMeal-Q (P =0 .19). Thiamine, folate, magnesium and fiber intake were similar between the WFR and Meal-Q (P = 0.16-0.92). There were no differences in intakes between Meal-Q and MiniMeal-Q for any nutrients (P < 0.001). (Wilcoxon's signed-rank test).

[b]n = 162 due to exclusion of one subject with implausibly high intake.

[c]n = 160 due to exclusion of three subjects with implausibly high intakes.

Table 3 shows the quartile cross-classifications between Meal-Q, MiniMeal-Q and the WFR for energy, macro- and micronutrients as well as the quartile cross-classification between the questionnaires and the DLW method. For comparisons with the WFR regarding energy, the proportion classified into the same/adjacent quartile was 70% for Meal-Q and 67% for MiniMeal-Q. The proportions for macronutrients were 76-86% for both questionnaires. For micronutrients assessed with Meal-Q, 69-90% of the subjects were classified into the same/adjacent quartile and for MiniMeal-Q, it ranged from 67% to 89%. Cross-classification of Meal-Q and DLW placed 77% into the same/adjacent quartile, and the proportion was the same for MiniMeal-Q.

Table 3. Quartile cross-classification of mean daily energy and energy-adjusted[a] macro- and micronutrient intake assessed with Meal-Q, MiniMeal-Q and the WFR (n=163), and cross-classification of mean daily energy intake and energy expenditure measured with DLW (n=39).

	Same quartile		Adjacent quartile		Extreme quartile	
	Percent of subjects (%)					
	Meal-Q	**MiniMeal-Q**	**Meal-Q**	**MiniMeal-Q**	**Meal-Q**	**MiniMeal-Q**
Energy (kJ)	26	27	44	40	9	7
Protein (g)	36	40	40	36	7	6
Carbohydrate (g)	42	42	40	37	3	2
Total fat (g)	37	33	41	46	8	9
Saturated fat (g)	52	45	33	37	5	4
Monounsaturated fat (g)	44	44	33	33	6	7
Polyunsaturated fat (g)	33	31	47	49	6	5
Alcohol (g)	50	49	36	37	4	4
Beta-carotene (µg)[b]	41	41	40	42	4	6
Thiamine (mg)	27	31	44	43	7	5
Riboflavin (mg)	37	36	37	40	4	4
Niacin (mg)	36	34	45	45	4	5
Vitamin B6 (mg)	34	31	41	45	6	6
Folate (µg)	42	40	38	42	4	4
Vitamin B12 (µg)	44	39	34	33	5	4
Vitamin C (mg)	39	38	46	45	2	3
Vitamin D (µg)	36	35	40	40	7	6
Vitamin E (mg)	40	42	34	33	4	4
Calcium (mg)	36	35	38	36	7	9
Iron (mg)	38	37	41	40	4	5
Magnesium (mg)	42	39	40	44	3	3
Phosphorus (mg)	33	34	42	43	7	7
Potassium (mg)	36	37	42	42	5	6
Selenium (µg)	41	38	37	42	4	6
Sodium (mg)[c]	33	35	36	32	10	11
Zinc (mg)	34	33	43	43	7	7
Fiber (g)	53	55	37	34	1	3
DLW, energy (kJ)	33	33	44	44	3	3

[a] Adjustments for total energy intake were made using the residual method[73].

[b] n = 162 due to exclusion of one subject with implausibly high intake.

[c] n = 160 due to exclusion of three subjects with implausibly high intakes.

Table 4 shows the Pearson and Spearman correlation coefficients between Meal-Q, MiniMeal-Q and the WFR as well as the DLW method. The de-attenuated correlations for energy and macronutrients ranged from r=0.18 to r=0.73 for Meal-Q and from r=0.18 to r=0.74 for MiniMeal-Q. De-attenuated correlations for micronutrients and fiber intake for Meal-Q ranged from r=0.31 to r=0.69 (excluding the statistically non-significant correlation for sodium) and the

correlations were very similar for MiniMeal-Q. Correlations with DLW was r=0.42 for Meal-Q and r=0.38 for MiniMeal-Q.

Table 4. Pearson and Spearman correlation coefficients[a] between Meal-Q, MiniMeal-Q and the WFR (n=163) as well as DLW (n=39).

	Crude[b]		Energy-adjusted[b,c]		De-attenuated (95% CI)[d]	
	Meal-Q	**MiniMeal-Q**	**Meal-Q**	**MiniMeal-Q**	**Meal-Q**	**MiniMeal-Q**
Energy	0.16	0.16	-	-	0.18 (0.01-0.36)	0.18 (0.01-0.33)
Protein	0.22	0.21	0.30	0.31	0.33 (0.17-0.47)	0.34 (0.18-0.48)
Carbohydrates	0.54	0.54	0.62	0.57	0.65 (0.54-0.74)	0.60 (0.48-0.70)
Total fat	0.06	0.02	0.55	0.49	0.57 (0.45-0.67)	0.51 (0.37-0.62)
Saturated fat	0.15	0.11	0.57	0.54	0.60 (0.48-0.70)	0.57 (0.44-0.67)
Monounsaturated fat	0.13	0.08	0.52	0.46	0.56 (0.43-0.67)	0.50 (0.36-0.62)
Polyunsaturated fat	0.23	0.21	0.36	0.35	0.42 (0.25-0.56)	0.40 (0.23-0.54)
Alcohol	0.64	0.65	0.61	0.63	0.73 (0.59-0.82)	0.74 (0.60-0.83)
Beta-carotene[e]	0.51	0.51	0.46	0.46	0.51 (0.36-0.64)	0.51 (0.36-0.64)
Thiamine	0.33	0.37	0.28	0.35	0.35 (0.16-0.52)	0.43 (0.24-0.59)
Riboflavin	0.16	0.15	0.39	0.38	0.42 (0.27-0.55)	0.41 (0.26-0.54)
Niacin	0.39	0.37	0.43	0.42	0.47 (0.32-0.59)	0.46 (0.31-0.59)
Vitamin B6	0.39	0.40	0.32	0.32	0.35 (0.19-0.49)	0.35 (0.19-0.49)
Folate	0.50	0.50	0.50	0.50	0.53 (0.39-0.64)	0.53 (0.39-0.64)
Vitamin B12	0.39	0.28	0.46	0.37	0.51 (0.36-0.63)	0.41 (0.26-0.55)
Vitamin C	0.53	0.52	0.53	0·50	0.57 (0.36-0.64)	0.54 (0.41-0.65)
Vitamin D	0.34	0.32	0.31	0.30	0.36 (0.19-0.50)	0.34 (0.18-0.49)
Vitamin E	0.30	0.30	0.42	0.42	0.48 (0.32-0.61)	0.48 (0.33-0.61)
Calcium	0.23	0.22	0.29	0.24	0.31 (0.16-0.45)	0.25 (0.09-0.40)
Iron	0.44	0.43	0.42	0.38	0.46 (0.31-0.59)	0.42 (0.27-0.55)
Magnesium	0.52	0.52	0.54	0.52	0.56 (0.44-0.66)	0.54 (0.41-0.64)
Phosphorus	0.36	0.37	0.36	0.36	0.39 (0.24-0.52)	0.39 (0.24-0.52)
Potassium	0.42	0.42	0.41	0.38	0.43 (0.29-0.56)	0.40 (0.25-0.52)
Selenium	0.32	0.30	0.42	0.41	0.45 (0.30-0.57)	0.44 (0.30-0.57)
Sodium[f]	0.32	0.32	0.15	0.12	0.16 (-0.01-0.32)	0.14 (-0.04-0.30)
Zinc	0.33	0.34	0.31	0.31	0.34 (0.18-0.49)	0.35 (0.19-0.49)
Fiber	0.66	0.65	0.67	0.65	0.69 (0.60-0.77)	0.67 (0.57-0.75)
DLW, energy (CI)[g]	0.42 (0.16-0.68)	0.38 (0.10-0.66)			-	

[a] Pearson correlation coefficients was used for energy and macronutrient intake and Spearman correlation coefficients were used for micronutrients and fiber intake.

[b] All correlation coefficients were statistically significant (P = 0.00-0.046), except for crude total, saturated and monounsaturated fat for both questionnaires (P = 0.06-0.84).

[c] Adjustments for energy were made using the residual method[73].

[d] De-attenuated values were obtained using the formula suggested by Beaton et al[74] and Liu et al[75]. Confidence intervals were produced using the method suggested by Willett and Rosner[76].

[e] n = 162 due to exclusion of one subject with implausibly high intake.

[f] n = 160 due to exclusion of three subjects with implausibly high intakes.

[g] Confidence intervals were obtained using the bootstrap method[77].

Figure 6 illustrates the Bland-Altman plots on energy for the WFR, Meal-Q and MiniMeal-Q in comparison with the DLW method, and **Figure 7-10** shows the plots for Meal-Q and the WFR for energy, macro- and micronutrients. In Figure 6, the plots indicated that the WFR and both questionnaires underestimated energy intake for most subjects. Meal-Q and MiniMeal-Q showed larger under-estimation of intake than the WFR and a trend of decreasing accuracy with increasing intakes. The plots for energy and macronutrients, comparing Meal-Q to the WFR, revealed a negative mean difference for energy and all macronutrients (Figure 7). For energy and polyunsaturated fat, there was a trend of decreasing accuracy with increasing intakes, and for other macronutrients, there was an increasing underestimation with increasing intakes. Regarding micronutrients, niacin was largely under-estimated by Meal-Q (approximately 20 mg) (Figure 8). Most of the micronutrients showed increasing under-estimation with increasing intakes and some also had a trend of increasing variance at higher intakes (Figure 8-10). In contrast, fiber had a larger variance at lower compared to higher intakes. Zinc, magnesium, potassium and phosphorus showed less varying bias over the intake range. The proportion of subjects outside the limits of agreement deviated somewhat from 5% for some plots due to varying bias over the intake range. The Bland-Altman plots for MiniMeal-Q and the WFR were very similar to those for Meal-Q (see **Appendix 10.1.1-10.1.4**).

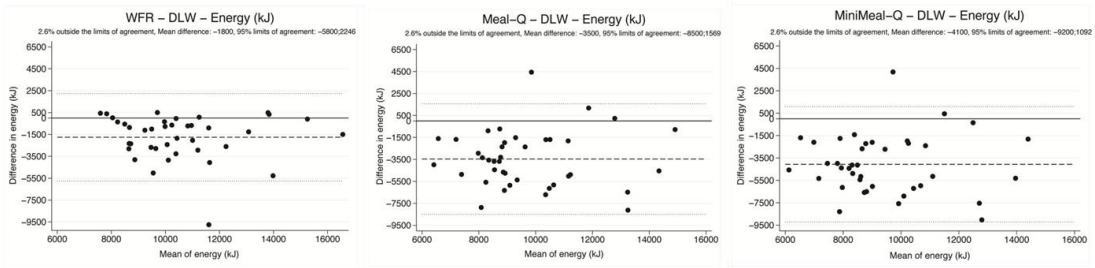

Figure 6. Bland-Altman plots showing the differences in energy intake assessed with the WFR, Meal-Q and MiniMeal-Q and the energy expenditure measured with DLW plotted against the mean of the two methods (n=39). Each data point represents one subject. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD).

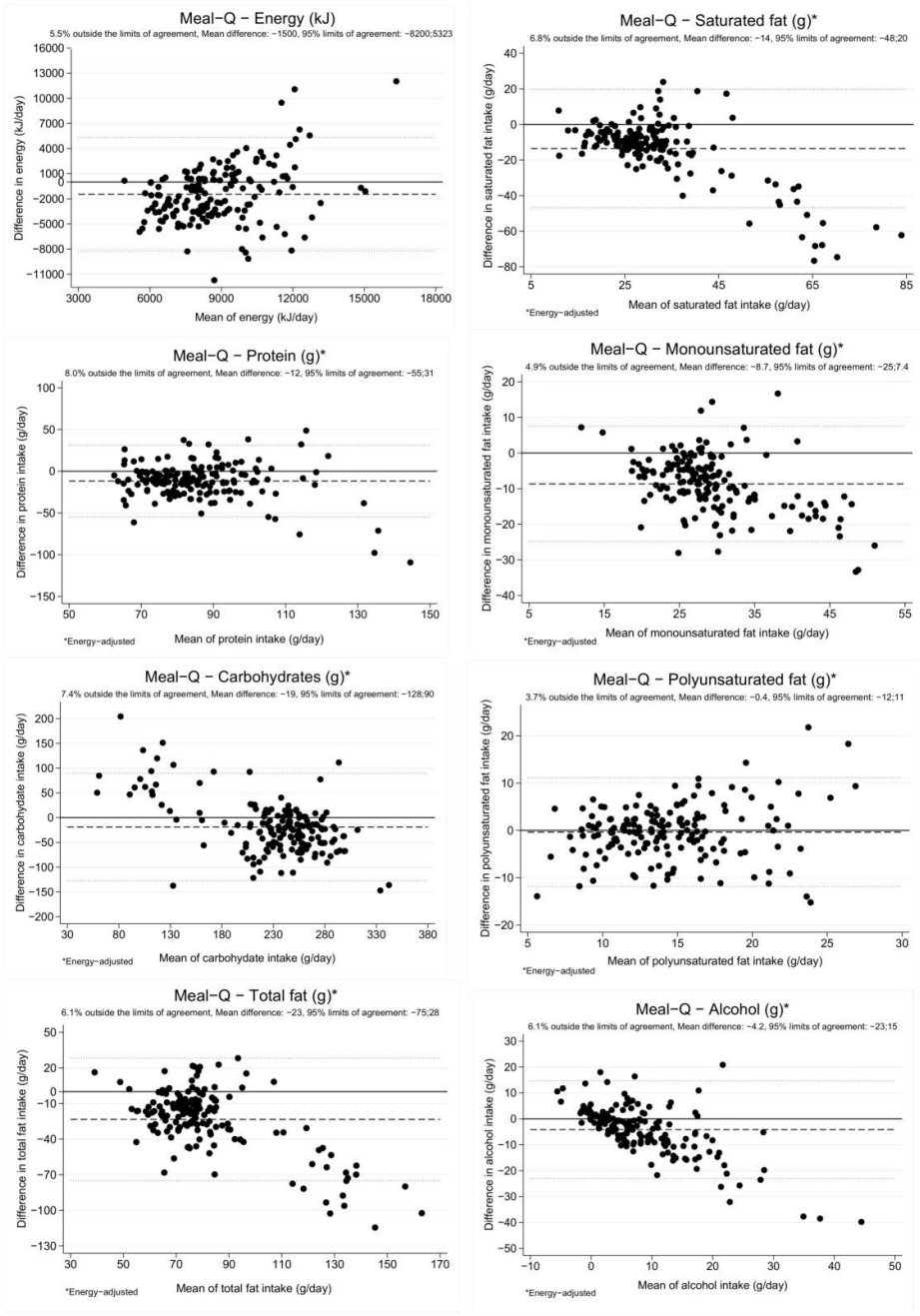

Figure 7. Bland-Altman plots showing the differences in energy, protein, carbohydrate, total fat, saturated fat, monounsaturated fat, polyunsaturated fat and alcohol intake assessed with Meal-Q and the WFR plotted against the mean of the two methods (n=163). Each data point represents one subject. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). Macronutrients are energy-adjusted.

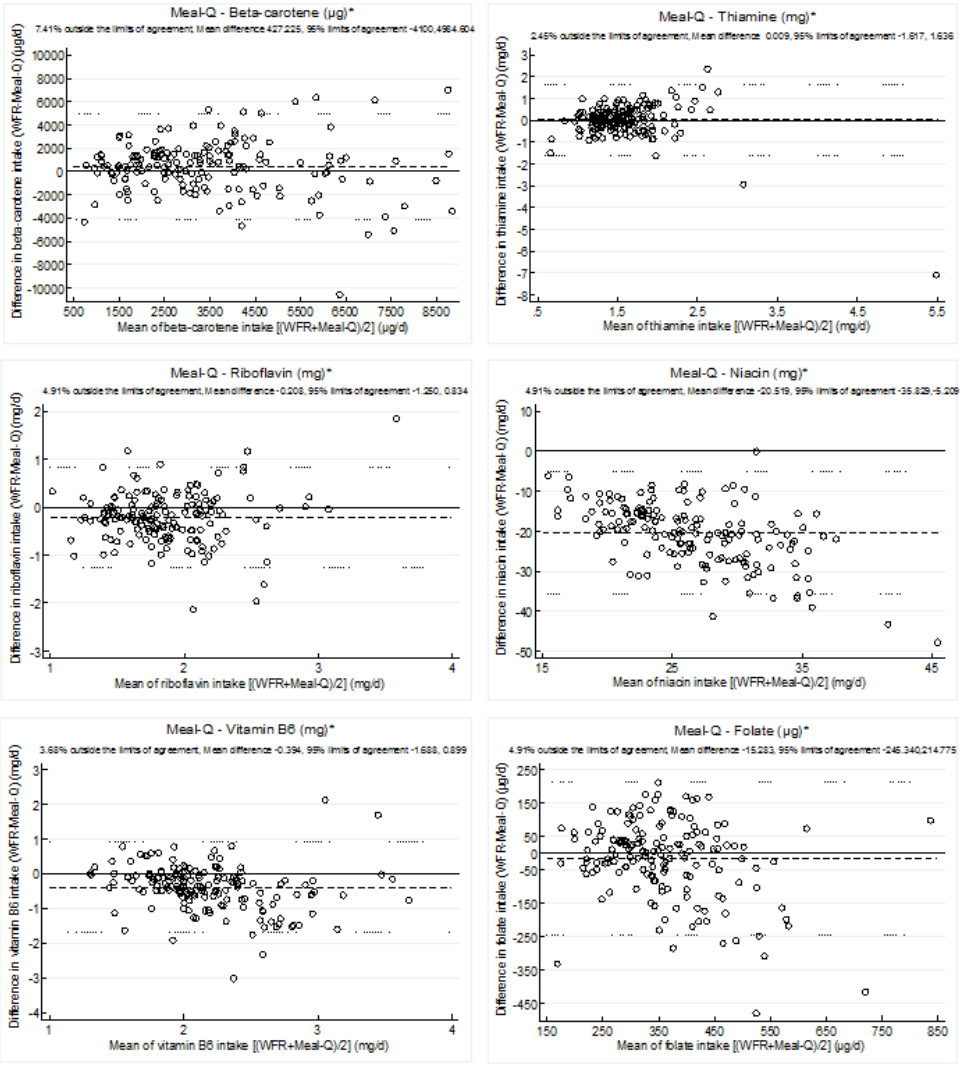

Figure 8. Bland-Altman plots with the WFR and Meal-Q for beta-carotene (n=162 (due to exclusion of one subject with implausibly high intake)), thiamine, riboflavin, niacin, vitamin b6 and folate (n=163). Differences in intake between the WFR and Meal-Q are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). *Energy-adjusted.

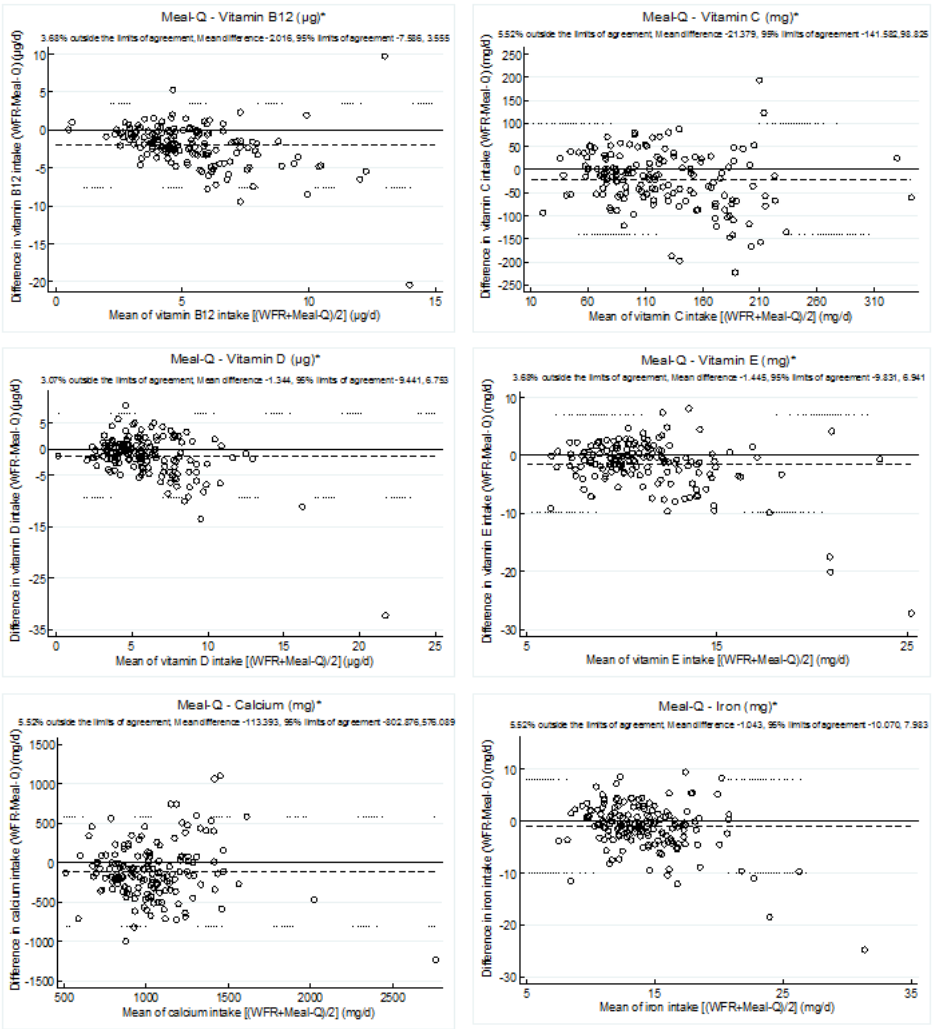

Figure 9. Bland-Altman plots with the WFR and Meal-Q for vitamin B12, vitamin C, vitamin D, vitamin E, calcium and iron (n=163). Differences in intake between the WFR and Meal-Q are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). *Energy-adjusted.

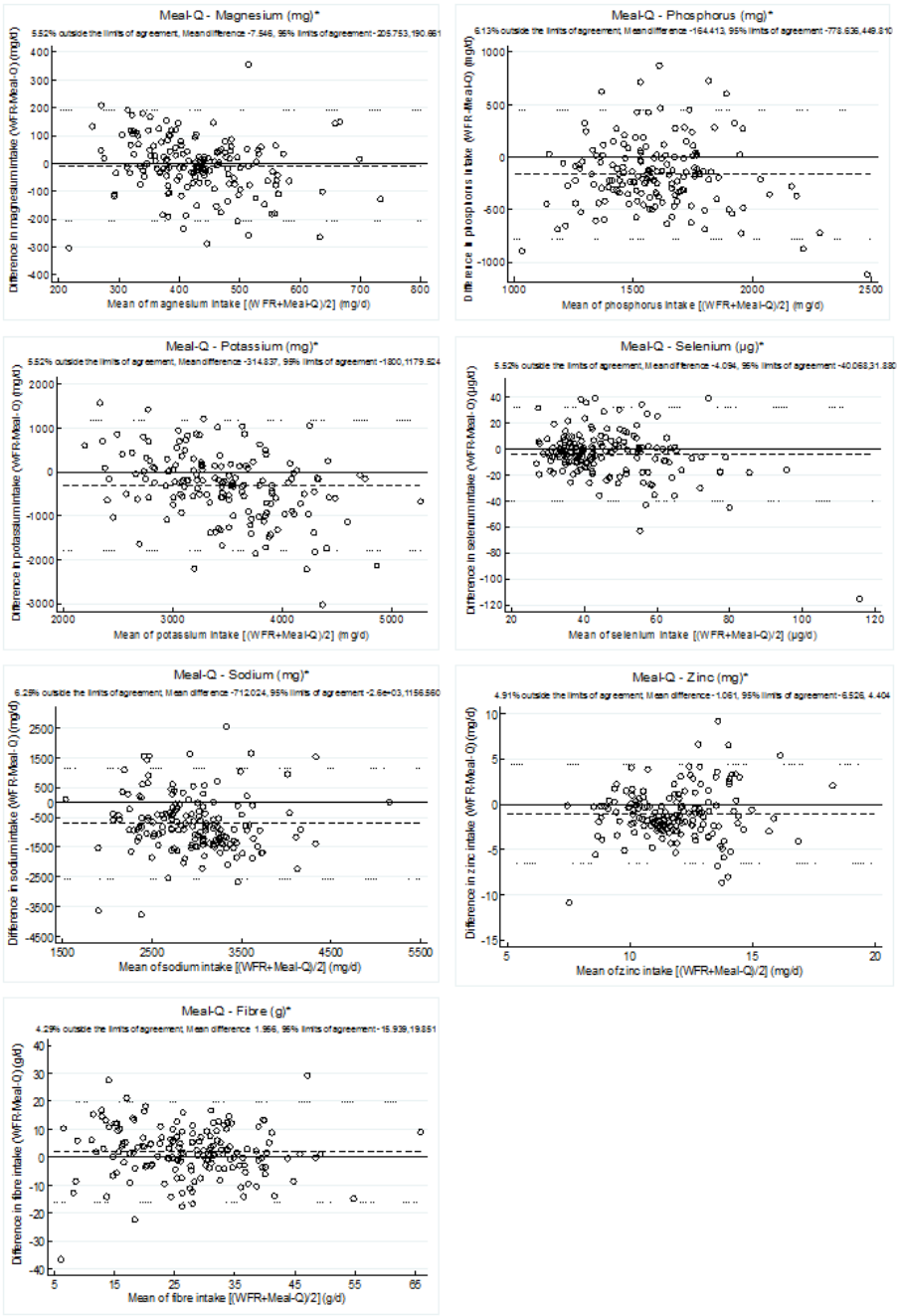

Figure 10. Bland-Altman plots with the WFR and Meal-Q for magnesium, phosphorus, potassium, selenium sodium (n=160 due to exclusion of three subjects with implausibly high intakes), zinc and fiber (n=163). Differences in intake between the WFR and Meal-Q are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). *Energy-adjusted.

5.1.3 Reproducibility

The comparison between Meal-Q1 and Meal-Q2 showed no statistically significant difference in crude intakes (P = 0.07-0.96). **Table 5** shows the quartile cross-classifications of the two Meal-Q assessments as well as the intra-class correlation coefficients (ICC). For energy and macronutrient intake, the proportion of subjects classified into the same/adjacent quartile ranged 85-96%. For micronutrient and fiber intake, the corresponding proportion was 86-97%. The ICCs for energy and energy-adjusted macronutrient intake ranged from r=0.57 to r=0.90, and the ICCs for energy-adjusted micronutrient and fiber intake ranged from r=0.50 to r=0.80.

Table 5. Quartile cross-classifications of Meal-Q1 and Meal-Q2 as well as crude and energy-adjusted[a] intra-class correlation coefficients[b] (ICC) (n=87)[c].

	Same quartile	Adjacent quartile	Extreme quartile	ICC (95% CIs)	
	Percent of subjects (%)			Crude	Energy-adjusted
Energy (kJ)	51	34	7	0.57 (0.42-0.71)	-
Protein (g)	53	40	2	0.52 (0.37-0.67)	0.73 (0.63-0.83)
Carbohydrates (g)	52	41	2	0.64 (0.51-0.76)	0.67 (0.56-0.80)
Total fat (g)	59	26	6	0.47 (0.30-0.63)	0.57 (0.43-0.71)
Saturated fat (g)	61	25	3	0.43 (0.26-0.60)	0.58 (0.44-0.72)
Monounsaturated fat (g)	56	32	3	0.50 (0.34-0.66)	0.60 (0.46-0.73)
Polyunsaturated fat (g)	57	36	3	0.65 (0.53-0.77)	0.68 (0.56-0.79)
Alcohol (g)	74	22	1	0.92 (0.89-0.95)	0.90 (0.87-0.94)
Beta-carotene (µg)	53	43	1	0.85 (0.79-0.91)	0.75 (0.66-0.84)
Thiamine (mg)	57	34	2	0.54 (0.40-0.69)	0.64 (0.51-0.76)
Riboflavin (mg)	59	33	3	0.45 (0.28-0.62)	0.63 (0.51-0.76)
Niacin (mg)	52	41	0	0.66 (0.54-0.78)	0.76 (0.67-0.85)
Vitamin B6 (mg)	53	36	1	0.49 (0.33-0.65)	0.50 (0.34-0.66)
Folate (µg)	59	38	1	0.71 (0.60-0.81)	0.73 (0.63-0.83)
Vitamin B12 (µg)	59	31	1	0.60 (0.47-0.74)	0.65 (0.53-0.78)
Vitamin C (mg)	52	43	1	0.80 (0.73-0.88)	0.74 (0.64-0.83)
Vitamin D (µg)	43	43	3	0.56 (0.42-0.70)	0.55 (0.41-0.70)
Vitamin E (mg)	57	34	0	0.73 (0.64-0.83)	0.73 (0.63-0.83)
Calcium (mg)	51	38	1	0.49 (0.33-0.65)	0.66 (0.54-0.78)
Iron (mg)	51	41	2	0.61 (0.47-0.74)	0.61 (0.48-0.74)
Magnesium (mg)	66	31	1	0.64 (0.51-0.76)	0.73 (0.64-0.83)
Phosphorus (mg)	54	33	3	0.46 (0.29-0.62)	0.62 (0.49-0.75)
Potassium (mg)	56	38	0	0.65 (0.52-0.77)	0.80 (0.73-0.88)
Selenium (µg)	61	33	1	0.64 (0.52-0.77)	0.72 (0.61-0.82)
Sodium (mg)	57	38	1	0.53 (0.38-0.68)	0.59 (0.45-0.72)
Zinc (mg)	46	40	1	0.50 (0.35-0.66)	0.63 (0.50-0.76)
Fiber (g)	55	39	0	0.77 (0.69-0.86)	0.71 (0.61-0.82)

[a]Adjustments for energy were made using the residual method[73].

[b]Intraclass correlation coefficients were computed using 1-way ANOVA with random effects[21].

[c]Missing values on Meal-Q 2 for 4 participants.

5.2 PROSPECTIVE COHORT STUDY – LIME

Paper III – *Adherence to the Nordic Nutrition Recommendations as a measure of a healthy diet and upper respiratory tract infection*

5.2.1 Subject characteristics and descriptive results

Table 6 shows the baseline characteristics of the study participants in the LIME study by adherence to the NNR. There were only small differences between the adherence groups regarding age, sex, BMI, asthma, energy intake and macronutrient intake. A higher adherence to NNR was associated with lower perceived stress, lower education level and fewer smokers.

Table 6. Characteristics of the study participants (n=1350) in the LIME study by adherence to the Nordic Nutrition Recommendations (NNR).

	Adherence to the NNR (initial scoring model) (score 0 – 6)		
	Low Adherence	**Medium Adherence**	**High Adherence**
	<4.5 points	**4.5 – 5.5 points**	**>5.5 points**
No of participants (%)	488 (36)	687 (51)	175 (13)
Age (years); %			
20-29	33	31	29
30-39	26	27	21
40-49	19	19	24
50-60	22	23	27
Sex (male); %	41	47	47
Body mass index; %			
Low-normal (<25 kg/m^2)	56	56	60
High-very high (>25 kg/m^2)	44	44	40
Chronic stress level; %			
Low (<23, below median)	47	55	58
High (>23, above median)	53	45	42
Education; %			
Secondary school or less	37	37	46
University	63	64	55
Smoking; %			
Current smokers	18	14	11
Previous smokers	31	28	22
Never smokers	32	58	67
Asthma (yes); %	7	8	11
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Energy intake (kJ/d);	7261 (2582)	7801 (2101)	7834 (1559)
Carbohydrate intake (g/d);	224 (84)	244 (66)	251 (50)
Protein intake (g/d);	75 (33)	81 (26)	80 (17)
Saturated fat intake (g/d);	21 (9)	23 (8)	23 (7)

Regarding each dietary recommendation, only 7% of the participants were within recommended level for PUFA intake (5-10% of total energy intake) and only 8% reached the recommended intake of vitamin D (7.5µg/d). In addition, for intake of retinol equivalents, α-tocopherol and selenium, only 17, 13 and 16% reached the recommended intake levels. For remaining nutrients and for physical activity, the percentage reaching the recommendations was 27-99% and 79%, respectively.

5.2.2 Nordic Nutrition Recommendations and URTI

No association could be found between adherence to each individual group of recommendations and URTI (**Table 7**).

Table 7. Adherence score (0-1) for each individual group of recommendations of the Nordic Nutrition Recommendations (NNR) and risk of upper respiratory tract infection.

		Adjusted for age and sex			Multivariable model	
Categorization of adherence score for each group of recommendation of the NNR[a]	**Cases**	**Person-weeks**	**IRR**	**95% CI**	**IRR[b]**	**95% CI**
Fat group[c] intake						
(adherence score, 0-1)						
Low (<0.50)	267	3457.5	1.00	-	1.00	-
Med (0.50-0.60)	487	6898.5	0.97	0.84-1.13	0.96	0.82-1.12
High (>0.60)	404	6354	0.92	0.78-1.08	0.91	0.78-1.08
Vitamin group intake						
(adherence score, 0 – 1)						
Low (<0.60)	414	5772	1.00	-	1.00	-
Med (0.60-0.90)	539	7918.5	0.98	0.86-1.12	0.93	0.78-1.10
High (>0.90)	205	3019.5	1.05	0.89-1.25	0.97	0.77-1.24
Mineral group intake						
(adherence score, 0 – 1)						
Low (<0.75)	277	4045.5	1.00	-	1.00	-
Med (0.75-0.95)	558	7938	1.07	0.93-1.24	1.08	0.90-1.30
High (>0.95)	323	4726.5	1.11	0.94-1.30	1.08	0.85-1.37
Sodium intake						
(adherence score, 0-1)						
Low (<0.80)	288	3870	1.00	-	1.00	-
Med (0.80-<1.00)	252	3645	0.96	0.81-1.13	0.97	0.80-1.18
High (1.00)	618	9195	0.92	0.80-1.06	0.93	0.74-1.17
Fiber intake						
(adherence score, 0-1)						
Low (<0.50)	245	3994.5	1.00	-	1.00	-
Med (0.50-1.00)	450	6432	1.00	0.86-1.17	0.97	0.82-1.14
High (1.00)	463	6883.5	1.03	0.88-1.20	0.96	0.80-1.16

[a]Carbohydrates (including sugar), protein, physical activity and alcohol were not included in this table since >75% of participants were within the recommended level (adherence score = 1) for these groups.

[b]IRR for all adherence scores were adjusted for age (20-29, 30-39, 40-49, and 50-60 years), sex, energy intake (in four categories), BMI (low, normal, overweight, and obese), weakened immune system (yes/no), asthma (yes/no), perceived stress (below and above median), education level (secondary school or less and university), smoking (daily/less frequent/previous/never) and month (February to May).

[c]Including total fat intake, saturated fat intake, essential fat intake, polyunsaturated fat intake, and monounsaturated fat intake.

According to the initial scoring model, 50% of the study participants had medium adherence (4.5-5.5 points) and 13% had high adherence (>5.5 points) to the NNR. High adherence to the NNR (>5.5 points) was not associated with a lower risk of URTI compared to low adherence (>4.5 points) (IRR 0.89, 95% CI 0.73-1.08). When testing different scoring models the IRR (95% CI) did not change considerably (model 1: 0.91 (0.76-1.09); model 2: 0.89 (0.74-1.08); model 3: 0.91 (0.76-1.09)).

Table 8. Absolute cut-off points for individual recommendations of the Nordic Nutrition Recommendations (NNR) and risk of upper respiratory tract infection.

		Adjusted for age and sex			Multivariable model	
Intake of individual recommendations of the NNR[a]	**Cases**	**Person-weeks**	**IRR**	**95% CI**	**IRR[b]**	**95% CI**
Saturated fat intake (% of energy)						
<10	315	4636.5	1.00	-	1.00	-
10-12	393	5485.5	1.07	0.92-1.24	1.09	0.93-1.27
>12	450	6588	1.03	0.89-1.19	1.05	0.90-1.22
Protein intake (% of energy)						
<17	421	5815.5	1.00	-	1.00	-
17-20	533	7615.5	1.02	0.89-1.16	1.02	0.89-1.16
>20	204	3279	0.94	0.79-1.12	0.94	0.78-1.11
Carbohydrate intake (% of energy)						
<50	195	2806.5	1.00	-	1.00	-
50-60	725	10780.5	0.93	0.79-1.09	0.92	0.78-1.09
>60	238	3123	0.98	0.80-1.19	0.96	0.78-1.17
Sodium intake (mg/d)						
<2000	313	4258.5	1.00	-	1.00	-
2000-3800	661	9925.5	0.96	0.83-1.10	0.87	0.71-1.06
>3800	184	2526	1.01	0.83-1.23	0.85	0.63-1.14
Alcohol intake (% of energy)						
<2.5	586	8094	1.00	-	1.00	-
2.5-5.0	328	4896	0.99	0.86-1.14	0.99	0.86-1.14
>5.0	244	3720	1.02	0.88-1.19	1.05	0.87-1.12
Fiber intake (g/d)						
<25	695	9826.5	1.00	-	1.00	-
25-35	302	4518	1.02	1.17-1.04	0.99	0.86-1.15
>35	161	2365.5	1.04	0.88-1.24	0.98	0.80-1.19
Moderate[c] and/or vigorous physical activity (min/d)						
<60	522	7062	1.00	-	1.00	-
60-120	281	3940.5	0.90	0.78-1.05	0.89	0.77-1.05
>120	378	5982	0.86	0.74-1.01	0.82	0.69-0.97

[a]Single vitamins or minerals were not studied since they were too many in number and would increase the risk of chance findings.

[b]IRR for all adherence scores were adjusted for age (20-29, 30-39, 40-49, and 50-60 years), sex, energy intake (in four categories), BMI (low, normal, overweight, and obese), weakened immune system (yes/no), asthma (yes/no), perceived stress (below and above median), education level (secondary school or less and university), smoking (daily/less frequent/previous/never) and month (February to May).

[c]Moderate physical activity cut-offs: <2 h, 2-3 h, >3 h

Table 8 shows the absolute cut-off points for the individual recommendations of the NNR and the risk of URTI. We found no association between adherence for any individual recommendation and URTI except for physical activity that was associated with a reduced risk of URTI (IRR 0.82, 95% CI 0.69-0.97). Due to the large number of nutrients and the risk of chance findings, single vitamins and minerals were not included in the analysis of individual recommendations.

Figure 11 shows the graphs of the cubic spline regressions with the initial scoring model for overall adherence to the NNR and URTI as well as for moderate and vigorous physical activity (hours/d) and URTI. Overall adherence to the NNR showed no association with URTI, whereas a trend of lower URTI rate could be seen for increased hours of moderate and vigorous physical activity.

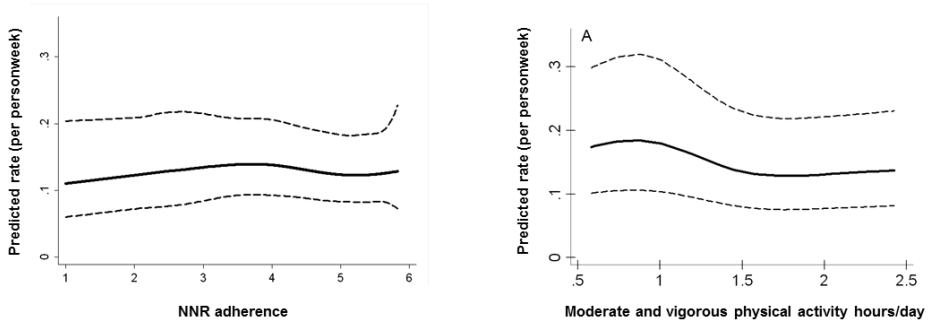

Figure 11. Cubic spline regression for overall adherence to the Nordic Nutrition Recommendations (NNR) as well as for moderate and vigorous physical activity (hours/d) and smoothed incidence rates of upper respiratory tract infection.

5.3 PROSPECTIVE COHORT STUDY – SWEDE-I

Paper IV – *Intake of vitamin C, vitamin E, selenium, zinc and polyunsaturated fatty acids and upper respiratory tract infection: a prospective cohort study*

5.3.1 Subject characteristics and descriptive results

Table 9 shows the baseline characteristics of the study subjects in the SWEDE-I study. There were small differences between women and men, yet more men than women were overweight, whereas women were more likely to have a university degree than men. Women reported a somewhat higher crude vitamin C intake than men, while men reported a slightly higher crude selenium and zinc intake.

Table 9. Characteristics of study subjects.

	All subjects, n=1533	Women, n=926	Men, n=607
Age (years); %			
25-34	12	13	11
35-44	32	32	32
45-54	27	29	25
55-64	29	27	32
Body Mass Index[a]; %			
Normal weight	45	52	35
Overweight	36	27	48
Obese	14	14	15
Education[b]; %			
Upper secondary school or less	40	32	52
University	59	67	48
Current smokers[c]; %	11	12	8.9
Asthma[d]; %	9.8	11	7.9
Immunodeficiency disease /reduced immune defence[e]; %	1.3	1.4	1.2
Chronic illness[f]; %	20	19	21
Physical activity level (PAL); Median (IQR)	1.80 (1.54;2.07)	1.82 (1.57; 2.05)	1.77 (1.48; 2.14)
Energy and nutrient intake[g]; Median (IQR)			
Energy intake; kJ	6820 (5271;9038)	6543 (5146; 8570)	7201 (5693; 9681)
Vitamin C intake; mg	69 (44;103)	73 (47; 105)	68 (40; 100)
Vitamin E intake; mg	7.8 (5.7;10.5)	7.9 (5.7; 10.6)	7.7 (5.7; 10.2)
Selenium intake; mcg	35 (26;46)	34 (26; 44)	37 (28; 48)
Zinc intake; mg	9.2 (7.2;12.2)	8.9 (7.0; 11.8)	9.7 (7.5; 12.8)
Omega-6 intake; g	0.70 (0.41;1.22)	0.70 (0.40; 1.20)	0.69 (0.42; 1.23)
Arachidonic acid intake; mg	58 (40;80)	57 (39; 79)	59 (42; 82)
Omega-3 intake; g	0.23 (0.12;0.46)	0.23 (0.12; 0.46)	0.22 (0.12; 0.46)
EPA intake; mg	49 (33;104)	45 (33; 104)	52 (34; 105)
DHA intake; g	0.18 (0.11;0.32)	0.17 (0.11; 0.32)	0.18 (0.11; 0.32)

[a]Body Mass Index categories, normal weight: <25; overweight: 25-30; obese: >30. One man and 10 women were underweight (BMI <18.5). Missing information on BMI for 8 men and 58 women.

[b]Missing information on education for 5 men and 6 women.

[c]Defined as smoking daily during the last month. Missing information on smoking for 2 men and 3 women.

[d]Asthma treated by a doctor (yes/no). Missing information on asthma for 13 men and 19 women.

[e]Immunodeficiency disease or reduced immune system treated by a doctor (yes/no). Missing information for 16 men and 22 women.

[f]Chronic illness (heart disease, high blood pressure, diabetes, rheumatism, kidney disease or tumor) treated by a doctor (yes/no). Missing information on chronic illness for 22 men and 30 women.

[g]Not adjusted for energy intake.

Table 10 shows the mean (SD) daily energy-adjusted nutrient intake from food categorized into low, medium and high intake as well as frequency of supplement intake. Daily or weekly consumption of vitamin C supplements was more frequent among women than men, whereas intake of multivitamin/minerals and omega-3 fatty acids was similar between the sexes.

The number of URTI events per subject ranged from 0-8 with a mean of 0.87. Women reported URTI more frequently than men (1.02 vs. 0.71). The URTI incidence peaked in September-October followed by a decline and increased again in mid-December to reach a second peak in mid-February. The lowest incidence of URTI was reported in May at the end of follow-up.

5.3.2 Antioxidants and polyunsaturated fatty acids and URTI

Table 11 and **12** shows the nutrient intake from food, the number of cases/1000 person-weeks and IRR (95% CI) for URTI among all subjects. The multivariate adjusted models (IRR (95% CI)) for women showed a reduced risk of URTI for vitamin C (0.69 (0.55-0.88)), vitamin E (0.77 (0.62-0.96)) and DHA (0.57 (0.39-0.83)), a borderline protective effect for selenium (0.78 (0.61-1.01)) and AA (0.80 (0.65-0.99)), when comparing high to low intake. No association was seen for zinc (0.88 (0.66-1.17)), omega-6 (0.87 (0.70-1.07)) or EPA (0.90 (0.73-1.10)). Among men, we saw no inverse association for any nutrient. In contrast, we found an increased risk for URTI among men with medium vitamin E intake (1.42 (1.09-1.85)) and high zinc intake (1.50 (1.04-2.16)).

The proportional hazards assumption was not violated for any model. Moreover, effect measure modification by other nutrient intake or interaction with time could not be found for any nutrient. Since pollen allergy can mimic URTI symptoms, we considered a model excluding subjects with pollen allergy. The estimates changed somewhat for vitamin C, vitamin E and AA, while estimates for other nutrients remained the same. When evaluating if the date that the participants filled out MiniMeal-Q could have affected the reporting, the results did not changed for any of the nutrient.

Table 10. Mean (SD) daily intake of nutrients[a] from food categorized as low, medium and high intake as well as supplement use categorized as daily/weekly, monthly and sporadic use.

	Women, n=926						Men, n=607					
	Low Intake; Mean (SD)	n	Medium intake; Mean (SD)	n	High intake; Mean (SD)	n	Low Intake; Mean (SD)	n	Medium intake; Mean (SD)	n	High intake; Mean (SD)	n
Vitamin C, mg	33.1 (8.4)	193	74 (18)	552	158 (57)	172	27.6 (8.7)	160	66 (17)	325	140 (57)	113
Vitamin E, mg	5.6 (0.80)	233	7.8 (0.81)	481	11.5 (2.4)	203	5.2 (0.80)	145	7.1 (0.71)	340	10.2 (2.2)	113
Selenium, µg	19.4 (4.8)	188	34.2 (5.5)	551	59 (15)	187	19.0 (5.2)	164	31.9 (4.2)	295	50 (12)	146
Zinc, mg	6.4 (1.1)	200	9.1 (0.95)	551	13.0 (2.3)	175	5.8 (1.3)	125	8.4 (0.85)	332	11.5 (1.5)	148
Omega-6 fatty acids, g	0.31 (0.10)	200	0.78 (0.23)	521	2.1 (1.0)	205	0.30 (0.10)	122	0.70 (0.21)	343	2.3 (1.5)	142
Arachidonic acid intake; mg	30 (7.6)	224	60 (12)	512	116 (37.8)	189	28 (9.1)	140	59 (11)	338	116 (40.7)	129
Omega-3 fatty acids, g	0.082 (0.032)	174	0.27 (0.10)	571	0.85 (0.46)	181	0.082 (0.034)	136	0.24 (0.082)	339	0.95 (0.67)	132
EPA; mg	0.015 (0.010)	213	0.054 (0.019)	490	0.14 (0.06)	223	0.012 (0.010)	136	0.053 (0.017)	327	0.16 (0.10)	144
DHA; g	0.06 (0.03)	175	0.19 (0.06)	539	0.46 (0.18)	212	0.06 (0.03)	133	0.19 (0.06)	353	0.50 (0.20)	121
Supplement use; n (%)	**Daily/weekly**		**Monthly**		**Sporadic**		**Daily/weekly**		**Monthly**		**Sporadic**	
Vitamin C	49 (5.3)		18 (1.9)		80 (8.6)		20 (3.3)		19 (3.1)		29 (4.8)	
Multivitamin/mineral	124 (13)		27 (2.9)		81 (8.7)		81 (13)		15 (2.5)		34 (5.6)	
Omega-3 fatty acids[b]	101 (11)		6 (0.6)		43 (4.6)		54 (8.9)		7 (1.2)		23 (3.8)	

[a]Energy-adjusted using the residual method[73].

[b]Fish oil.

Table 11. Intake of nutrients[a] from food and risk of upper respiratory tract infection among all subjects (n=1533).

	Crude			Crude model[b]		Multivariate model[c,d]	
	Cases	Person-weeks	Cases/1000 Person-weeks	IRR	95% CI	IRR	95% CI
Vitamin C (mg/d)							
Women							
<45	196	16568	12	1.00	-	1.00	-
45-110	612	49859	12	1.06	0.90-1.24	1.04	0.86-1.24
≥110	142	13718	10	0.73	0.59-0.91	0.69	0.55-0.88
Men							
<40	101	10980	9	1.00	-	1.00	-
40-100	262	25469	10	1.21	0.96-1.52	1.19	0.92-1.53
≥100	65	8039	8	0.80	0.59-1.10	0.81	0.59-1.12
Vitamin E (mg/d)							
Women							
<6.5	222	18772	12	1.00	-	1.00	-
6.5-9.5	529	43453	12	1.03	0.88-1.21	0.99	0.84-1.17
≥9.5	199	17920	11	0.83	0.69-1.01	0.77	0.62-0.96
Men							
<6	73	8798	8	1.00	-	1.00	-
6-8.5	268	26240	10	1.39	1.07-1.80	1.42	1.09-1.85
≥8.5	87	9451	9	1.22	0.90-1.67	1.26	0.91-1.74
Selenium (µg/d)							
Women							
<25	191	15483	12	1.00	-	1.00	-
25-45	593	49304	12	0.98	0.83-1.16	1.03	0.84-1.26
≥45	166	15358	11	0.76	0.62-0.94	0.78	0.61-1.01
Men							
<25	102	10831	9	1.00	-	1.00	-
25-40	229	22648	10	1.13	0.89-1.42	1.15	0.87-1.52
≥40	97	11009	9	0.90	0.68-1.18	0.94	0.67-1.30
Zinc (mg/d)							
Women							
<7.5	186	15571	12	1.00	-	1.00	-
7.5-11	612	50308	12	0.99	0.84-1.17	1.05	0.85-1.31
≥11	152	14266	11	0.75	0.61-0.93	0.88	0.66-1.17
Men							
<7	59	7319	8	1.00	-	1.00	-
7-10	255	25297	10	1.40	1.05-1.85	1.47	1.07-2.01
≥10	114	11872	10	1.30	0.95-1.77	1.50	1.04-2.16

[a]Energy-adjusted using the residual method[73].

[b]n=1533

[c]All multivariate models were adjusted for age, sex, BMI, education and energy intake. Zinc was additionally adjusted for dietary selenium intake.

[d]n=1459, 74 subjects with missing values, whereof 62 women and 12 men.

Table 12. Intake of nutrients[a] from food and risk of upper respiratory tract infection among all subjects (n=1533).

	Crude			Crude model[b]		Multivariate model[c,d]	
	Cases	Person-weeks	Cases/1000 person-weeks	IRR	95% CI	IRR	95% CI
Omega-6 (g/d)							
Women							
<0.45	204	16966	12	1.00	-	1.00	-
0.45-1.25	532	44912	12	0.95	0.81-1.12	0.93	0.78-1.11
≥1.25	214	18267	12	0.93	0.77-1.13	0.87	0.70-1.07
Men							
<0.4	68	7926	9	1.00	-	1.00	-
0.4-1.2	265	26393	10	1.30	1.00-1.70	1.26	0.94-1.71
≥1.2	95	10169	9	1.16	0.85-1.58	1.12	0.79-1.58
Arachidonic acid (mg/d)							
Women							
<40	245	20077	12	1.00	-	1.00	-
40-85	522	43924	12	0.93	0.80-1.08	0.95	0.80-1.12
≥85	183	16144	11	0.81	0.67-0.99	0.80	0.65-0.99
Men							
<40	92	9943	9	1.00	-	1.00	-
40-80	249	25075	10	1.12	0.88-1.42	1.11	0.86-1.44
≥80	87	9470	9	0.99	0.74-1.32	0.96	0.70-1.32
Omega-3 (g/d)							
Women							
<0.13	152	13526	11	1.00	-	1.00	-
0.13-0.5	606	50668	12	1.13	0.94-1.35	1.11	0.91-1.34
≥0.5	192	15951	12	1.11	0.90-1.38	1.01	0.80-1.29
Men							
<0.13	92	9812	9	1.00	-	1.00	-
0.13-0.45	256	25495	10	1.04	0.82-1.32	1.05	0.80-1.39
≥0.45	80	9182	9	0.84	0.63-1.13	0.83	0.58-1.18
EPA (mg/d)							
Women							
<30	221	18411	12	1.00	-	1.00	-
30-100	498	42278	12	0.95	0.80-1.12	0.95	0.80-1.13
≥100	231	19456	12	0.90	0.74-1.09	0.90	0.73-1.10
Men							
<25	80	9208	9	1.00	-	1.00	-
25-100	254	24991	10	1.26	0.98-1.63	1.26	0.95-1.68
≥100	94	10289	9	1.04	0.77-1.40	1.04	0.75-1.44
DHA (g/d)							
Women							
<0.1	193	15539	12	1.00	-	1.00	-
0.1-0.32	556	46989	12	0.93	0.79-1.09	0.81	0.61-1.07
≥0.32	201	17617	11	0.81	0.67-0.99	0.57	0.39-0.83
Men							
<0.1	80	9086	9	1.00	-	1.00	-
0.1-0.35	270	26739	10	1.23	0.96-1.57	1.06	0.76-1.49
≥0.35	78	8664	9	1.02	0.75-1.39	0.71	0.45-1.11

[a]Energy-adjusted using the residual method[73].

[b]n=1533

[c]All multivariate models were adjusted for age, sex, BMI, education and energy intake. DHA was additionally adjusted for dietary EPA intake.

[d]n=1459, 74 subjects with missing values, whereof 62 women and 12 men.

We found no association between daily or weekly supplement use of vitamin C and/or multivitamin/mineral or omega-3 and URTI incidence. The estimates were similar when excluding subjects with pollen allergy as well as when adjusting for date of filling out MiniMeal-Q.

Figure 12 depicts the restricted cubic spline regressions for dietary intake of vitamin C, vitamin E, zinc and DHA and URTI. The graphs showed similar results as found in the categorical analyses (Table 11 and 12). A trend of a decreasing IRR among men could be seen for increasing intake of vitamin C and DHA, if yet not statistically significant. In **Appendix 10.2.1**, graphs are illustrated for spline regressions on dietary intake of selenium, omega-6, AA, omega-3 and EPA. Although not statistically significant, the graphs showed trends of a decreasing IRR among men with increasing intake of omega-6, omega-3 and EPA. The same could be seen among women for increasing intake of selenium, omega-6, AA, omega-3 and EPA.

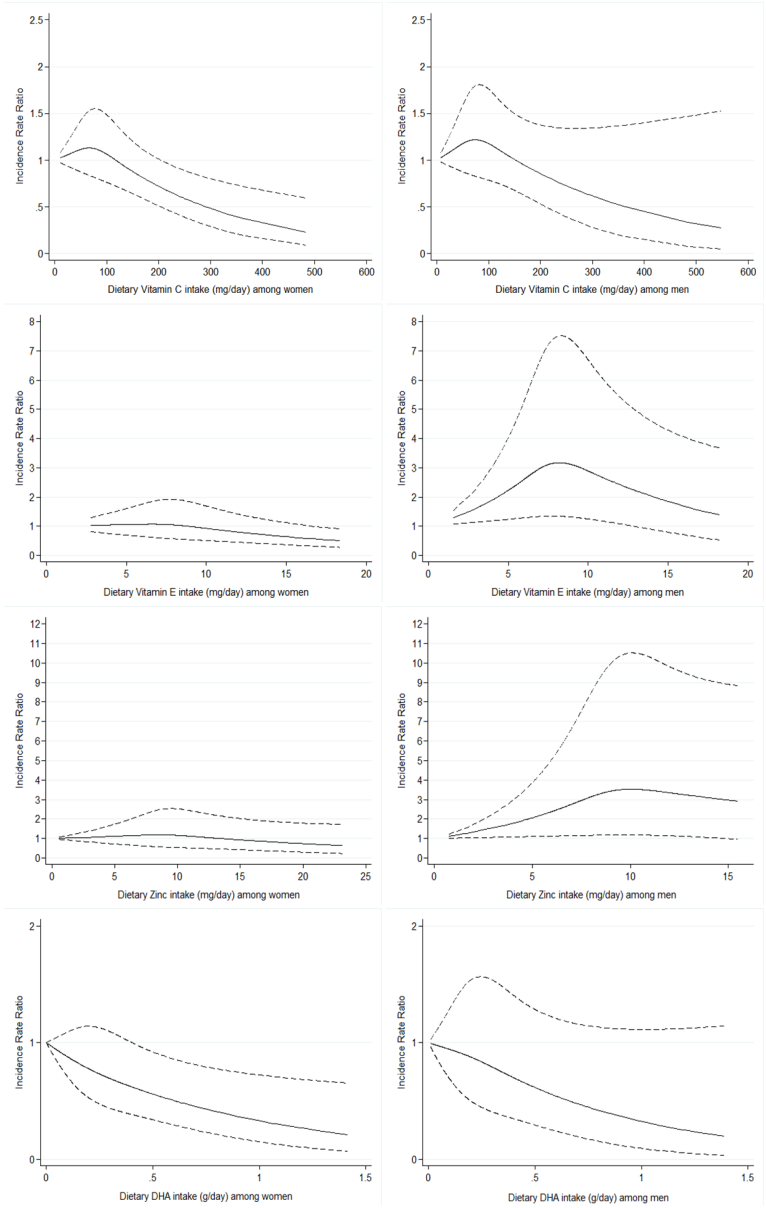

Figure 12. Restricted cubic spline regression models with smoothed incidence rate ratios (solid line) of URTI for dietary intake of vitamin C, vitamin E, zinc and DHA and 95% confidence intervals (dotted lines) displayed separately for women and men. All models were adjusted for age, energy, BMI and education. Zinc was additionally adjusted for dietary selenium intake and DHA was additionally adjusted for dietary EPA intake. The x-axis was truncated showing 5 to 95 percentile of the intake.

6 DISCUSSION

6.1 GENERAL METHODOLOGICAL CONSIDERATIONS

Accuracy

The accuracy of an epidemiological study is defined by the amount of systematic and random error inherent in the study. Systematic errors include selection bias, information bias and confounding, and are a threat to the validity of the study, whereas random errors affect the precision, i.e. with which certainty we can draw any conclusions from the results.

Selection bias

The presence of *selection bias* occurs if the association between exposure and disease outcome is different between those participating in a study compared to those who do not. However, since the association between exposure and disease outcome among non-participants is generally unknown, selection bias can only be assumed.

Misclassification of exposure and outcome

Misclassification, or measurement error of exposure is a type of *information bias* that can be non-differential or differential. If non-differential, the misclassification is unrelated to the outcome and the association is likely to be diluted if the outcome is dichotomous. On the other hand, when the misclassification is differential, it is related to the outcome and can lead to an over- or underestimation of the true association. Misclassification can also occur for the outcome and can be non-differential or differential with regards to the exposure. If it is non-differential, it normally generates a bias towards the null.

When it comes to measurement error in dietary intake assessment, there are several types[19]. One type is respondent biases, which arise from systematic under- or over-reporting of certain foods. This can be the result of a social desirability in wanting to report according to a certain *norm*. Generally, unhealthy foods tend to be under-reported, whereas healthy foods become over-reported. In this way, correlated measurement errors can also occur, i.e. when mis-reporting of one type of food product correlates with mis-reporting of another[96]. Furthermore, the respondents may have memory lapses, which result in unintentional systematic omission or addition of certain foods. A systematic incorrect estimation of both intake frequency and portion sizes can also occur. Besides error in reporting food intake, errors might arise in the coding process when frequency of intake is

transferred into nutrient intake. This can however be minimized with standardized assessment procedures and good quality-control.

Furthermore, measurement error can arise in the categorization of a continuous exposure, such as dietary intake. If possible, it is preferred to use biological or pre-set cut-offs. Otherwise, quantile cut-offs are often used. This can however create cut-off intervals with no meaningful exposure difference, i.e. the groups become too similar. By using cut-offs according to the exposure distribution, the groups can be chosen in a way that they represent varying levels of exposure [97]. To avoid misclassification of dietary intake by categorization, one can use spline regression where the exposure it let to be continuous[95].

Confounding

Confounding is the mixing of effects that occurs when the observed association between the exposure and the disease is affected by an external factor. This will lead to bias in the effect estimates and can result in an observed association where there is none or the masking of a true effect. Confounding is therefore affecting the validity of the study. A confounder is defined by affecting both the exposure and the disease and should not be in the causal pathway between them (**Figure 13**). Being in the causal pathway between exposure and disease is defined as being a mediator. It is important to control or adjust for confounding factors in epidemiological studies in order to enable an unbiased estimation of the true association between an exposure and the disease. If a covariate in itself is affected by the exposure and the disease, it is referred to as a collider. Adjustment for a collider should not be done as it opens up for an observed association that is erroneous.

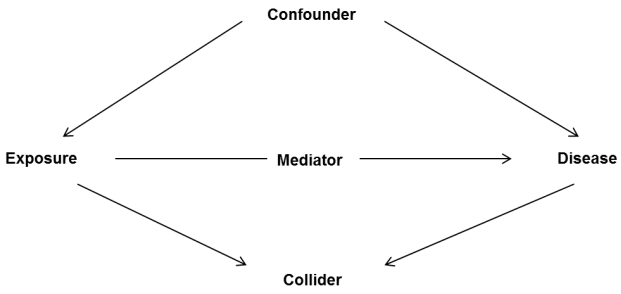

Figure 13. A directed acyclic graph (DAG) illustrating the concept of confounding.

There are different ways of dealing with confounding factors in epidemiological studies. It can be done at the design stage of a study, e.g. by randomization as in randomized controlled trials or restriction as in only including men in a study. In case-control studies, matching can be done on known confounders. In cohort studies, confounders can be dealt with at the analysis stage by stratification, which is efficiently done when using multivariate regression models where several

confounding factors can be adjusted for simultaneously. However, even after controlling for confounders, there might still be unknown or unmeasured confounders that were not evaluated and that would have affected the association. Residual confounding might also occur due to measurement errors in the confounding factors.

Random error

Random error in epidemiological studies leads to imprecise estimates, which results in less certain conclusions. Large amounts of random error means low precision and can for example be seen in a wide confidence interval. The confidence interval represents a range within which one is fairly certain that the true estimate lies and hence, if this is wide, the estimate should be interpreted with caution. The confidence interval is derived from the standard error, which is inversely related to the sample size. Therefore, random error decreases with increasing sample size. Random error in nutritional epidemiological studies can also be minimized by standardizing the assessment procedure and by including quality checks. Using a web-based FFQ as compared to a printed version is one way of reducing random error in dietary assessment since the administration process is more standardized and can include quality checks.

6.2 VALIDATION STUDY – VALMA

Paper I and **Paper II** are discussed jointly.

6.2.1 Main findings and interpretation

The results from the cross-classifications of Meal-Q, MiniMeal-Q and the WFR for energy, macro- and micronutrients placed 67-90% in the same/adjacent quartile. The cross-classifications with the DLW method placed 77% in the same/adjacent quartile for both questionnaires. Furthermore, the de-attenuated and energy-adjusted Pearson correlation coefficients between the questionnaires and the WFR ranged from r=0.33 to r=0.74 for macronutrients and was r=0.18 for energy. Correlations with DLW were r=0.42 for Meal-Q and r=0.38 for MiniMeal-Q. The de-attenuated and energy-adjusted Spearman correlations between the questionnaires and the WFR for micronutrients and fiber ranged from r=0.25 to r=0.69, with exclusion of sodium that was not statistically significant. Since sodium was not explicitly assessed in the questionnaires and the WFR and therefore only reflected salt already present in the food items and dishes from the Food Composition Table, the results should be interpreted with caution. The Bland-Altman plots showed on average large variances and a trend of increasing underestimation with increasing intakes for some nutrients. Regarding

reproducibility, the intra-class correlations for Meal-Q for energy-adjusted intakes ranged from *r*=0.50 to *r*=0.90.

The median answering time was 17 minutes for Meal-Q and 7 minutes for MiniMeal-Q. Despite their short answering time, both Meal-Q and MiniMeal-Q ranked most nutrient intake well compared to the reference methods and were considered highly user-friendly by the participants.

The high between-person variance captured by MiniMeal-Q as compared to Meal-Q indicates that it is possible to use a shorter questionnaire while still assessing a similar intake range and keeping the ranking ability. Because MiniMeal-Q originates from Meal-Q data and is also compared to the same reference methods, their results become highly related. Therefore, comparisons regarding assessments and relative validity should be made with caution.

The quartile cross-classification of energy and macronutrient with the WFR showed a fair ranking agreement for most of the nutrients for both Meal-Q and MiniMeal-Q, although a lower agreement was seen for energy and polyunsaturated fat. Similar rankings have been seen in three other validation studies of FFQs against food records[98-100] whereof one FFQ was web-based [98]. The cross-classifications of micronutrients and fiber with the WFR showed both questionnaires to yield ranking agreements comparable to or better than other similar validation studies[98,99,101-103], of which two evaluated web-based FFQs. The highest ranking agreement for Meal-Q and MiniMeal-Q was seen for fiber. The lowest ranking agreement was seen for sodium, as has been shown previously [98,99]. There are only a limited number of studies that have used Bland-Altman plots and therefore comparisons are difficult. However, the varying bias over the intake range seen in the plots has previously also been detected in two other studies regarding fiber, folate and iron[98,103].

High correlations between FFQs and diet records usually are in the order of 0.6-0.7 and it is unlikely to obtain correlations above 0.8[104]. A review of validation studies on FFQs concluded that the mean correlation between FFQs and food records of ≥6 days was 0.42 for energy, 0.57 for total fat, 0.53 for protein, 0.58 for carbohydrates, and 0.76 for alcohol[105]. In light of this literature, energy and protein seemed to perform less well, whereas other macronutrients showed correlations within expected ranges. In a review of 392 validation studies of vitamin intake, Henríquez-Sánchez *et al*. showed mean correlations between FFQs and dietary records in the range *r*=0.41-0.53[106]. Another review of 109 validation studies on iron, calcium, selenium and zinc reported mean correlations between a FFQ and a dietary record ranging from *r*=0.36 to *r*=0.60[107]. Both reviews show that the correlations for micronutrients in our study were in line with other validation studies for most nutrients, with the exception for thiamine, riboflavin,

vitamin B6, vitamin D, vitamin E, calcium and zinc, which correlations were somewhat lower in the VALMA study.

The DLW measurements showed that the WFR, Meal-Q, and MiniMeal-Q underestimated the energy intake by 17, 30, and 36%, respectively. Similar figures for food records and FFQs have been seen in other studies using the DLW method[108-110]. The correlation coefficients between the questionnaires and DLW were similar to a study by Andersen et al [111], but slightly lower than that of Kroke et al[112]. Despite the underestimation of energy intake and the large variance seen in the Bland-Altman plots, quartile cross-classification of the questionnaires with DLW showed a fair ranking agreement, similar to that found by Kroke et al[112].

The reproducibility of Meal-Q indicated that it performed well in its ability to rank participants according to their dietary intake, with a high proportion of subjects in the same/adjacent quartile and a low proportion of misclassified subjects. The quartile cross-classifications of micronutrients were comparable to Labonté *et al.*[98]. The ICCs for fiber, vitamin E, vitamin B6, niacin, vitamin C, beta-carotene, folate, magnesium and potassium were on average lower than those found by Schröder et al.[113]. Furthermore, the ICCs were slightly lower than the Pearson correlations found by Labonté *et al.*[98], yet higher than the Pearson correlations found by Pinto *et al.*[103]. Energy-adjusted correlations between repeated FFQs have generally ranged r=0.5-0.8 in other studies[19] and Meal-Q showed quite similar results.

6.2.2 Methodological considerations

Statistical methods

Correlation coefficients have been used extensively in validation studies but have been criticized since they only measure a relationship and not the agreement between two methods. The Bland-Altman method that evaluates absolute agreement between two methods has instead been recommended[20]. However, it should be noted that we would not expect an absolute agreement between a FFQ and a food record due to their inherent methodological differences. The plots are instead helpful in assessing the magnitude of the inaccuracy and to enable detection of potential varying bias over the intake range. In this context, correlation coefficients can be regarded as a complement by giving a measure of the linear relationship as well as the ranking ability.

Validity and precision

The Bland-Altman plots showed that Meal-Q and MiniMeal-Q had difficulties in precision for assessment of energy and many of the nutrients. For some nutrients, there was also an increasing under-estimation of intake with increasing intakes.

The plots with the DLW method clearly showed that the questionnaires under-estimated the energy intake for almost all participants. The large variance over the intake range and the increasing under-estimation with increasing intakes could arise from a limited frequency range of the questionnaires and/or limited number of food items. This can result in unintentional over-reporting among true low consumers and under-reporting among true high consumers. This feature can also be seen in a scatter plot and has been called the "flattened slope" effect[114]. However, a true small between-person variance in a population would also give similar results[19]. Unfortunately, we cannot be sure to what extent the limitations of the questionnaires and a potential small between-person variance explain our results.

In the validation of a dietary assessment method the reference method should have measurement errors independent from those of the test method. Since the WFR is an open-ended prospective method and a FFQ is a retrospective method with pre-defined food items and frequencies, measurement errors are less likely to be correlated. Nevertheless, both methods are, as all dietary assessment methods, susceptible to social desirability. This could affect them in similar ways and thereby increasing their error dependency. Therefore a validation study of a dietary assessment method should be viewed in terms of relative validity rather than absolute validity.

Ideally, the WFR should have been done repeatedly over a longer time period to better mirror the time period assessed in the questionnaires. Furthermore, the DLW measurement should preferably have been done repeatedly over a longer time period to reflect the habitual energy intake. However, time constraints made a longer validation study impossible. Nevertheless, when comparing dietary intake between the questionnaires and the WFR, adjustments for within-person variance in the WFR were made to minimize the effect of day-to-day variations in dietary intake.

Regarding reproducibility, true variations in dietary intake over time should be taken into account. Therefore, the repeated assessment should preferably be done in relation to the time frame of the FFQ. In the VALMA study, dietary intake assessed with Meal-Q reflected the past few months, while due to the time constraints, the second assessment was sent out after three weeks. Ideally, the second Meal-Q should have been sent out after 1-2 months.

Some assumptions are inherent with using the DLW method. The participants are assumed to be in energy balance, i.e. not to be losing or gaining weight during the measurement period. The participants in the VALMA study were recruited with the inclusion criterion that they should not be on a weight-loss diet. We also weighed the participants at the beginning and at the end of the study to confirm that no weight change had occurred. The isotopes in the DLW are assumed to only

mark water molecules and isotopes that leave the body are thought to do so only through water or carbon dioxide. Furthermore, the body water is assumed to be constant (in "steady state") and water that has left the body cannot re-enter. The respiratory quotient that is used is calculated based on the macronutrient composition of the diet. It is therefore crucial to choose a respiratory quotient that corresponds to the dietary composition of the study population. We used a respiratory quotient of 0.85, which is based on omnivores with 30-35% energy contribution from fat, which was in line with the intake in the VALMA population.

Strengths

The VALMA study included a large sample size for this type of validation study. Moreover, there was a low drop-out rate as well as high compliance for the assessment methods throughout the entire study. The high compliance probably reflects a well-motivated study population, something that is vital for the study's internal validity. Using the DLW method is an additional strength that enabled an objective estimation of total energy expenditure for the evaluation of energy intake. Regarding data handling, the web-based format of the questionnaires and the WFR minimized potential errors in the conversion of crude consumption data into nutrient intakes. Web-based questionnaires have been shown to improve data quality in previous studies [15,16].

External validity

It should be acknowledged that the young and mainly female study population of VALMA might have had implications on external validity. The notable proportion of participants with training in nutrition might also have affected the reported intake in being more accurate than would have been obtained with participants from the general population. This can have resulted in an over-estimation of the validity.

6.3 PROSPECTIVE COHORT STUDIES – LIME AND SWEDE-I

6.3.1 Main findings and interpretation

Paper III – *Adherence to the Nordic Nutrition Recommendations as a measure of a healthy diet and upper respiratory tract infection*

We found that the overall adherence to the NNR was moderately good. A high overall adherence to the NNR could not be associated with URTI as compared to low adherence. However, when analyzing individual recommendations of the NNR, we found that high physical activity was associated with a reduced risk of URTI.

This study is to our knowledge the first to investigate dietary recommendations in relation to URTI. Previous studies on dietary recommendations have focused on chronic diseases, i.e. cardiovascular disease and cancer[47-52]. However, physical activity has been linked to a decreased risk of URTI also in other studies[7,10,53-59,115] Fondell *et al*. found that high levels of physical activity were associated with an 18% reduced risk of URTI[10] and Nieman *et al*. found that physically active individuals reported 43% less days of URTI compared to those who were sedentary[59]. The acute effect of physical activity on the immune system includes an enhanced number of lymphocytes, natural killer cells and cytokines. Chronic exercise has also been shown to increase the resting activity of natural killer cells[116].

The absence of an association for overall adherence to the NNR and URTI in our study could be explained by the participants being generally well-nourished. They were skewed towards higher adherence scores for some recommendations, which lead to a limited variation in intake.

Paper IV – *Intake of vitamin C, vitamin E, selenium, zinc and polyunsaturated fatty acids and upper respiratory tract infection: a prospective cohort study*

This study showed that high dietary intake of vitamin C, vitamin E and DHA was associated with a reduced risk of URTI among women. There was also a suggestive inverse association of URTI among women with high intake of AA. No inverse association could be seen among men, instead we found an increased risk of URTI for medium vitamin E and high zinc intake from diet. We did not find any association between supplement intake and URTI among either women or men.

Our study is one of the few prospective cohort studies on dietary intake and URTI. Takkouche *et al*. did not find any association between the combined dietary and supplement intake of vitamin C or zinc and URTI[117]. However, in a previous report by our research group[118], Fondell *et al*. also found that high intake of dietary vitamin C among women was inversely associated with URTI, whereas no association could be seen among men. High intake of dietary vitamin E showed no association among either women or men. Regarding supplement intake, there was an inverse association among men for vitamin E as well as a suggestive inverse association for vitamin C, while no association could be seen among women for any supplement intake. Other studies on nutrient intake and URTI have mostly been randomized controlled trials on supplement intake conducted in specific populations, such as elderly, children and athletes. The outcome of these have showed a moderate risk reduction of URTI in the general population with intake of vitamin C supplements[29] as well as a reduced risk among children taking zinc supplements[32,33]. The results for vitamin E have so far been inconclusive[30,31]. To

our knowledge, no cohort studies or randomized controlled trials have studied the intake of selenium or PUFA in relation to URTI.

It is difficult to compare results from our cohort study and the randomized controlled trials due to the different study designs. Nutrient contents in supplements used in trials are generally much higher than what can be obtained from food. In addition, due to the differences in bioavailability and kinetics between dietary and supplement intake for various nutrients[119], there might also be different effects on the immune system.

We found large gender differences in our study. This might be explained by biological differences in nutrient metabolism and susceptibility to infections. The reaction to respiratory infections has previously been found to differ between men and women[22]. Nevertheless, considering the wide confidence intervals around the estimates for men compared to women, the interpretation of the results should be done with caution and further studies are needed to evaluate our findings.

6.3.2 Methodological considerations

Statistical methods

In **Paper III**, we chose to use the Poisson regression model to calculate incidence rate ratios. This model was suitable since we did not have information on the exact date of onset of URTI or its duration. We therefore assigned a person-time of 1.5 weeks to participants who reported an URTI event during a 3-week follow-up period. In this sense, we created time intervals for all participants for each of the 5 follow-up periods. We assumed that the rate of URTI did not change within each time interval. To investigate this, we fitted a generalized estimating equations (GEE) model, which accounts for the lack of independence among repeated events. We also considered a negative binomial model that does not assume every event to be independent. However, the results were very similar to the Poisson model. In **Paper IV**, we had information on the date of onset of URTI and therefore we chose to use the Cox regression model where person-time could be counted as finely as in days. The model was set to allow a subject to have multiple events during the course of the study. Regarding dependency between repeated events, we controlled for this by using the robust sandwich estimator for standard errors.

In both **Paper III** and **IV**, we used spline regression models to estimate a potential dose-response relationship between dietary intake and URTI. In this sense we could evaluate the exposure as continuous and compare it to the categorical analyses. The knots in a spline regression are freely chosen and have impact on the appearance of the curve. More knots will capture more variation

in the data and hence create a less smooth shape of the spline. If too many knots are included, the spline can become overfitted. One can evaluate the goodness of fit by testing different number of knots. If the shape of the spline changes systematically and dramatically, the fit is not good. On the other hand, if the change is unsystematic and small, the fit is good. In **Paper III**, we used 6 knots placed at quintile cut-offs as well as at minimum and maximum values within a set range. We also evaluated splines with other number of knots to see if the shape differed, yet all splines seemed to have a good fit. In **Paper IV**, we chose to put 3 evenly distributed knots. We evaluated the goodness of fit by increasing the number of knots and by placing 3 knots at minimum, median and maximum values, and for all these the results remained very similar to the initial splines.

Selection bias

In **Paper III,** the invited participants were randomly selected from the Swedish Total Population Registry and the initial response rate was 30%. It is therefore possible that mostly health-conscious individuals chose to participate. This could have limited the exposure range and is a possible explanation for the null findings for dietary recommendations. Nevertheless, the study population was by the time of the data collection comparable to the general Swedish population with regards to the prevalence of obesity, smoking and asthma [120,121] as well as the nutrient intake, which was in the same magnitude as in the national food consumption survey *Riksmaten* from 1997-1998[122]. The SWEDE-I study in **Paper IV** was a population-based study with individuals randomly selected from the Total Population Registry. However, since the overarching aim of the study was to investigate potential work-related risk factors for respiratory and gastrointestinal viral infections, a criterion for participation was to be employed. In addition, the initial response rate was 16%. The included individuals might therefore have been healthier than the general population and non-participants could have had a different diet that would affect the association with URTI differently. However, the study population was fairly similar to the general Swedish population on a number of variables, such as BMI, education and smoking[123-125]. Intake of nutrients were also fairly comparable to the national food consumption survey *Riksmaten* from 2010-2011[126], given that they used a food record, which assesses a larger part of the dietary intake compared to a FFQ. In **Paper III**, there was a high continuation rate for each of the five follow-ups (83-84%). The type of passive follow-up that was employed in **Paper IV**, which relies on self-initiated event-driven reporting, has previously been evaluated in a validation study that showed a participation rate of 86-88%[127]. For both **Paper III** and **IV**, we believe that the association between exposure and outcome did not differ between participants and non-participants and that the result could be generalized to the general population.

Misclassification of exposure

In both **Paper III** and **IV**, dietary intake was assessed with validated web-based FFQs. A FFQ does not assess the entire dietary intake, however epidemiological studies generally aim to rank the individuals according to their exposure and FFQs are suitable for this purpose. Nevertheless, a FFQ may be prone to measurement error such as the social desirability in under- and over-reporting certain foods. It might be that health-conscious individuals were affected by a social desirability in their reporting of diet and maybe also more prone to report an URTI, resulting in differential misclassification. This could result in a positive association between healthy dietary intake and URTI. In **Paper III**, we did not observe any such association. In **Paper IV** however, there was an inverse association between URTI and dietary intake of vitamin E and zinc among men. However, considering the wide confidence intervals, these results should be interpreted with caution. Non-differential misclassification of exposure might have occurred in both **Paper III** and **IV**, leading to an attenuation bias with dilution of the association as a result.

Misclassification of exposure can occur when creating diet quality scores. The score is for example dependent on its included components, grouping of foods/nutrients, choice of cut-offs and the weight of each component in the total score[128]. For each group of recommendation in **Paper III** (e.g. fat, carbohydrates, vitamins, and minerals), we summed up the scores for each individual recommendation and divided by the total number of individual recommendations included in the group. By doing this, we gave equal weight to each group in the final adherence score. We further used a continuous grading scale for intakes close to the NNR cut-offs, which reduced the problem of misclassification when choosing strict categorical scores.

The correlation coefficients in the validation studies of the FFQs used in **Paper III** and **IV** indicate that the dietary intake was assessed with some error. For the FFQ used in **Paper III**, the correlation coefficients with food records were moderate to high for different nutrients, ranging from r=0.31 for iron to r=0.81 for vitamin C. For MiniMeal-Q used in **Paper IV**, the correlation coefficients with weighed food records were r=0.54 for vitamin C, r=0.48 for vitamin E, r=0.44 for selenium, r=0.35 for zinc and r=0.40 for PUFA[83,84]. As is commonly the case with FFQs, the nutrients were somewhat under-estimated. There was also a varying bias over the intake range as seen in Bland-Altman agreement plots with the food records, which indicates random measurement error. Considering bias in the coding procedure of the FFQs, this was minimized by their web-based format with automatic checks for missing answers and standardized compiling of data. The spline regressions in both **Paper III** and **IV** showed similar results as the categorical analyses, indicating that the categories represented the different exposure levels well.

Misclassification of outcome

Misclassification of outcome may be a concern when it is self-reported. URTI was self-reported in both **Paper III** and **IV** and not validated with an objective method, hence misclassification is possible. However, we controlled for both pollen allergy and smoking, which can mimic URTI symptoms. When controlling for pollen allergy in **Paper IV**, we saw slight differences for some nutrients indicating that it might have had an effect on URTI classification. Misclassification could potentially also occur if symptoms of other illnesses were mistaken for URTI, e.g. angina that also gives a sore throat. However, it ought to be non-differential in relation to dietary intake. Under-reporting of URTI events has been documented in a previous study[127]. We cannot rule out that this was the case in our studies as well. If this is the case also in our studies and being it differential with regards to dietary intake, the estimates might have been affected.

Confounding

In **Paper III** and **IV**, confounding was dealt with in the regression models by including the confounding factors as covariates. In **Paper III**, the confounders were identified by backward deletion, i.e. by including all covariates of interest in the model and thereafter excluding one at a time to evaluate if the estimates changed. If they changed by more than 10%, the covariate was kept in the model. In **Paper IV**, a large number of potential confounding factors were evaluated and therefore, the approach of forward addition to the model was used. If the estimates changed with 10% or more, a covariate was kept in the model. In both **Paper III** and **IV**, sex, age, education, BMI and energy intake were included in the model although they showed only small confounding effects. This was done since these covariates have been identified in previous studies as potential confounders for the association between dietary intake and URTI. In **Paper III**, stress was included as a confounder. However, in **Paper IV** stress was not available as a covariate and could therefore not be adjusted for. Since stress has been shown to affect URTI[10,91] and possibly also affects dietary intake, it might be that the inclusion of this covariate would have changed the estimates. Other unmeasured confounders, e.g. unmeasured aspects of a healthy lifestyle or diet or unmeasured sources of antioxidants and PUFAs might also have affected the association. Measurement error in confounding variables, i.e. residual confounding, can be present in both **Paper III** and **IV**. Nevertheless, the effect ought to be minor in **Paper IV** due to the small number of identified confounders.

Strengths

Among the strengths of **Paper III** and **IV** are their population-based designs and large sample sizes. Both studies also have low possibility for reverse causation by

the prospective design in assessing dietary intake at baseline or early in follow-up as well as by the exclusion of individuals with URTI at baseline. Both studies used validated FFQs that were web-based, which likely minimized random measurement errors. In **Paper III**, the reporting of URTI was surveiled through frequent follow-ups. In **Paper IV**, we had the possibility to follow the study participants over a long time period, which covered the general URTI season in Sweden. Furthermore, in both studies, several potential confounding factors were available to adjust for, which minimizes bias in the estimates.

External validity

In **Paper III** and **IV**, the participants were selected through a randomized sample of the Swedish population. However, it might be that the individuals who chose to participate were different from those who did not. Furthermore, in **Paper IV**, a criterion for inclusion was to be employed. Nevertheless, the participants in both **Paper III** and **IV** were similar to the general Swedish population on a number of variables including BMI, education and smoking as well as dietary intake. A criterion for external validity is internal validity, which depends on a motivated study population with few drop-outs and high compliance, amongst other things. Given internal validity and that the association between exposure and URTI would not differ largely between participants and non-participants, we believe that the results can be generalized to the general population.

7 CONCLUSIONS AND FUTURE PERSPECTIVES

Paper I and II

- The validation study showed that Meal-Q and MiniMeal-Q had a short answering time of 17 and 7 minutes, respectively. Both were also regarded as highly user-friendly by the participants.
- Despite the short answering time, the ability of Meal-Q and MiniMeal-Q to rank individuals according to their dietary intake was good for most nutrients.
- The questionnaires had some difficulty in precision and showed increasing under-estimation with increasing intakes for some nutrients.
- MiniMeal-Q captured a high proportion (70-100%) of the between-person variance of Meal-Q and thereby proved that a shorter questionnaire has the ability to assess a similar intake range as Meal-Q.
- Meal-Q was found to have a good reproducibility.

Paper III

- The overall adherence to the Nordic Nutrition Recommendations was moderately good.
- A high overall adherence to the Nordic Nutrition Recommendations was not associated with risk of URTI.
- High physical activity was associated with an 18% reduced risk of URTI.

Paper IV

- High dietary intake of vitamin C, vitamin E and DHA among women was associated with a reduced risk of URTI by 31, 23 and 43%, respectively.
- No inverse association could be found among men. In contrast, medium intake of vitamin E and high intake of zinc from diet was associated with an increased risk of URTI of 42 and 50%, respectively.
- We found no association between supplement intake and risk of URTI among either women or men.

The use of web-based FFQs in epidemiology are likely to increase in the nearest future due to the increasing use of Internet and the advantages web-based questionnaires brings compared to printed versions in terms of cost-efficiency and data quality. Here, Meal-Q and MiniMeal-Q will be useful tools in assessing dietary intake. Both questionnaires are unique in the sense that they are interactive with follow-up questions based on previous answers. In this way the answering time can be kept down and the questionnaires get tailored to the

respondent's food habits. The meal-based design is likely to ease the recall of dietary intake and the validation study also showed that respondents found them highly user-friendly. Compared to a traditional food group-based non-interactive 72-item FFQ (Classic FFQ)[61] that was also evaluated in the VALMA study, MiniMeal-Q assesses up to 126 food items, yet still take the same average time to fill out (7 minutes).

Today, MiniMeal-Q is used in several ongoing cohort studies in Sweden including *LifeGene* (heredity, environment and lifestyle in relation to different health outcomes, www.lifegene.se), *KARMA* (breast cancer, karmastudy.org), *STHLM2* and *STHLM3* (prostate cancer, sthlm2.se, sthlm3.se), *EpiHealth* (lifestyle factors and gene interaction, www.epihealth.se) and *Scapis* (cardiopulmonary disease, www.sahlgrenska.se/sv/SU/Forskning/Centrum-for-klinisk-provning/SCAPIS). Together, these cohort studies will include several hundred thousand participants. The outcome of these studies will bring more knowledge on how dietary intake affects our most common diseases and potentially give suggestions for preventive actions. Furthermore, Meal-Q and MiniMeal-Q are also used in a number of smaller ongoing interventions and randomized controlled trials.

The relationship between physical activity and URTI was first suggested to be J-shaped, in which low and high levels of physical activity are associated with an increased risk of URTI, whereas moderate levels are associated with a reduced risk[129]. The relationship has thereafter been suggested to have an S-shape where the increased risk of high physical activity is followed by a reduced risk at elite levels[130]. There are to date few studies that have investigated the effect of moderate physical activity on URTI. Most previous studies have been conducted in small study populations and have focused on athletes. Hence, more cohort studies and experimental studies on the relationship between moderate physical activity and URTI are warranted.

If the inverse associations between vitamin C, vitamin E and DHA and URTI among women hold true and are causal, the individual burden and societal economic costs due to URTI could be reduced. However, more studies on dietary intake and URTI are needed to evaluate the reproducibility of our findings. In addition, the reasons for the strong interaction with gender warrant further studies. Future studies should consider combining dietary intake with a more detailed assessment of supplement intake. One could also evaluate biomarkers for dietary intake in relation to URTI and explore a potential relationship between dietary intake and different strains of viruses.

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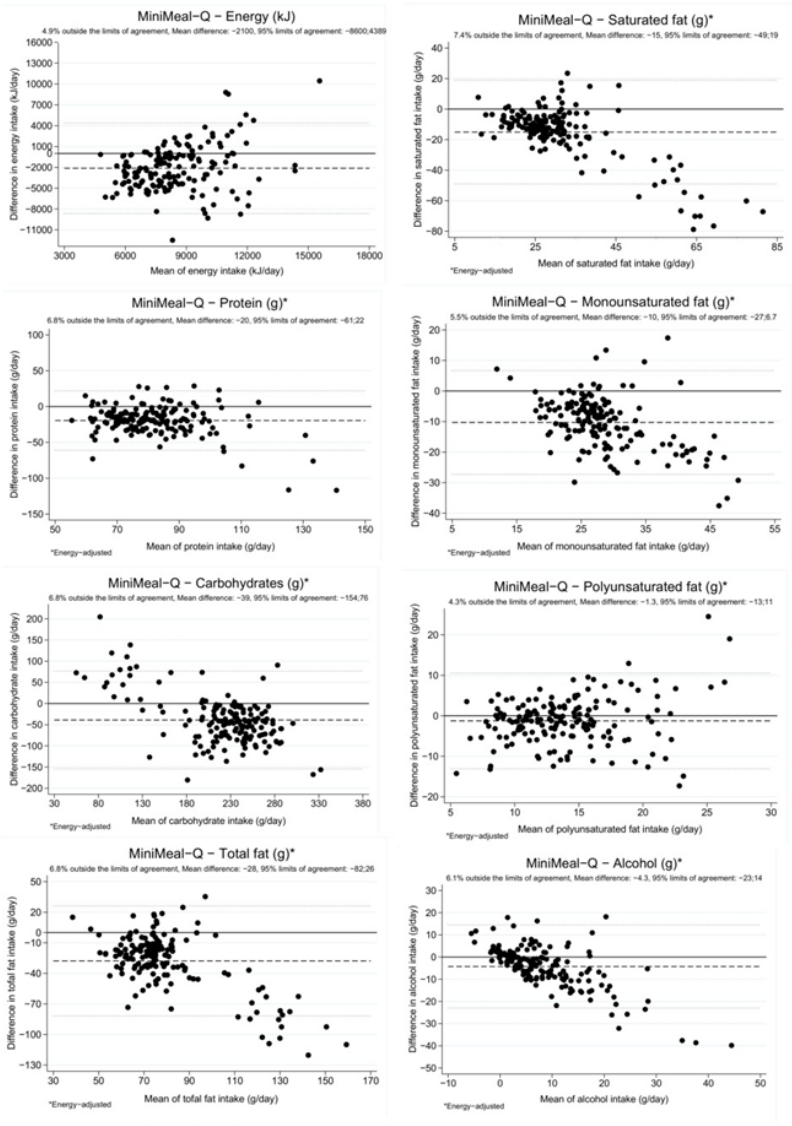
10 APPENDICES

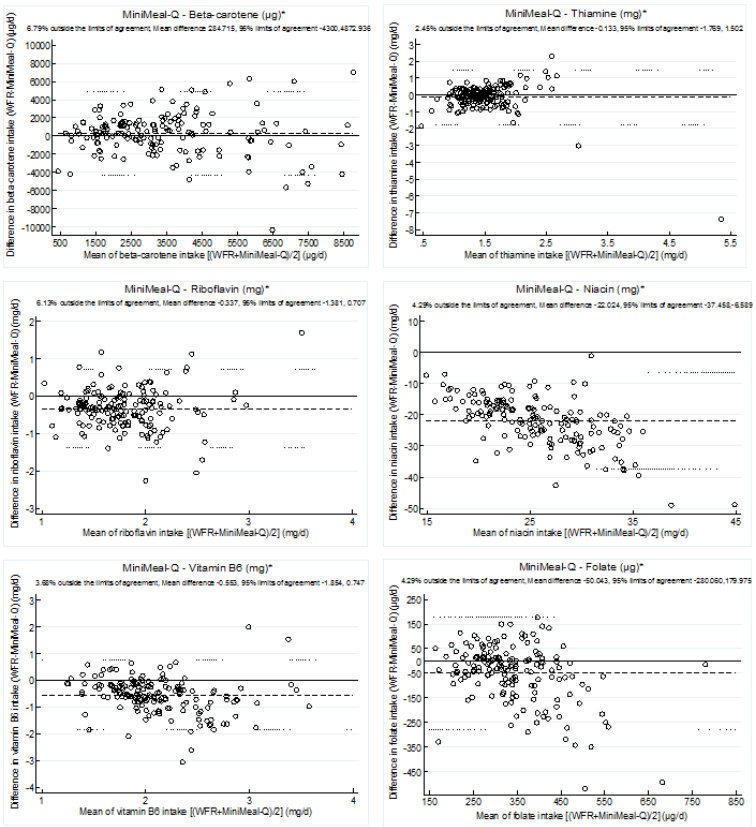
10.1 VALIDATION STUDY - VALMA

10.1.1 Appendix 1



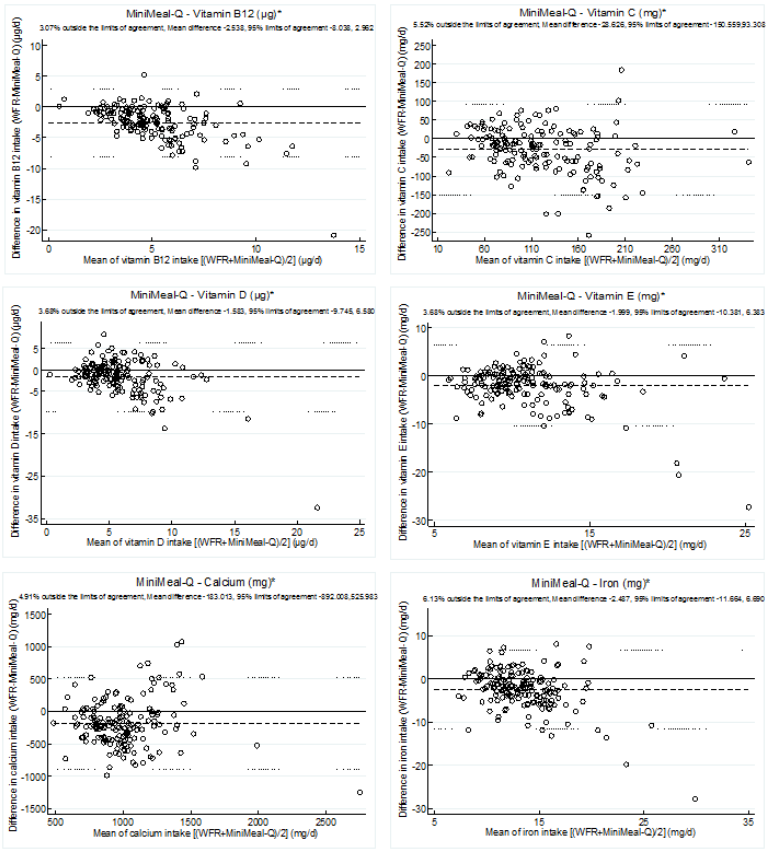
Appendix 1. Bland-Altman plots showing the differences in energy, protein, carbohydrate, total fat, saturated fat, monounsaturated fat, polyunsaturated fat and alcohol intake assessed with MiniMeal-Q and the WFR plotted against the mean of the two methods (n=163). Each data point represents one subject. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). Macronutrients are energy-adjusted.

10.1.2 Appendix 2



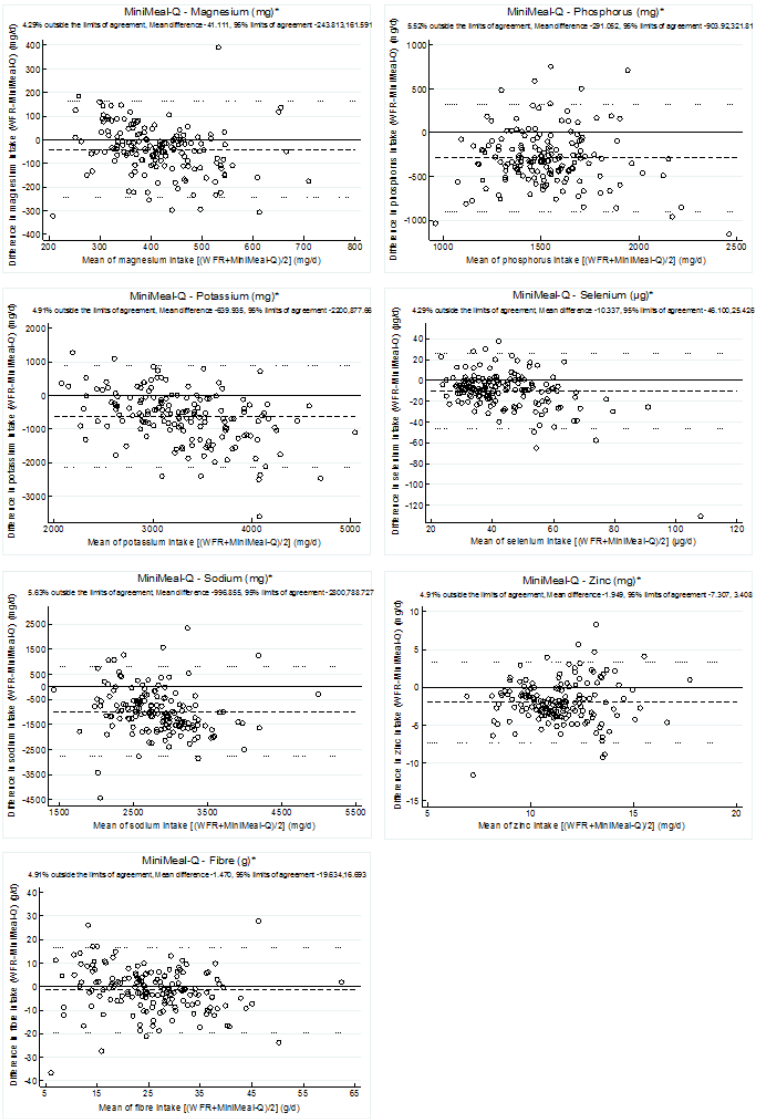
Appendix 2. Bland-Altman plots with the WFR and MiniMeal-Q for beta-carotene (n=162 (due to exclusion of one subject with implausibly high intake)), thiamine, riboflavin, niacin, vitamin b6 and folate (n=163). Differences in intake between the WFR and MiniMeal-Q are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). *Energy-adjusted.

10.1.3 Appendix 3



Appendix 3. Bland-Altman plots with the WFR and MiniMeal-Q for vitamin B12, vitamin C, vitamin D, vitamin E, calcium and iron (n=163). Differences in intake between the WFR and MiniMeal-Q are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). *Energy-adjusted.

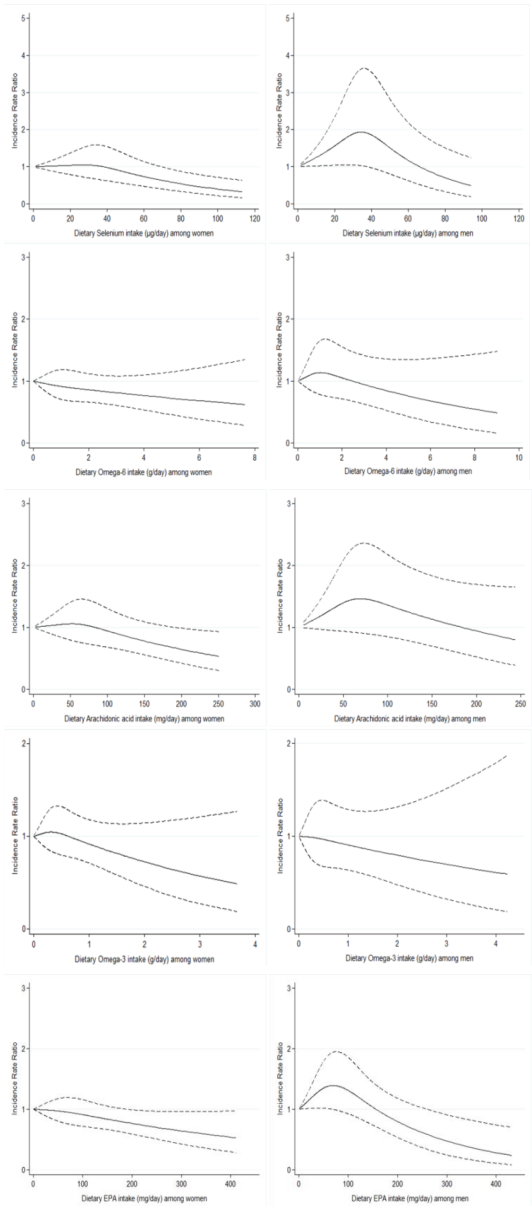
10.1.4 Appendix 4



Appendix 4. Bland-Altman plots with the WFR and MiniMeal-Q for magnesium, phosphorus, potassium, selenium sodium (n=160 due to exclusion of three subjects with implausibly high intakes), zinc and fiber (n=163). Differences in intake between the WFR and MiniMeal-Q are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). *Energy-adjusted.

10.2 PROSPECTIVE COHORT STUDY – SWEDE-I

10.2.1 Appendix 1



Appendix 1. Restricted cubic spline regression models with smoothed incidence rate ratios (solid line) of URTI for dietary intake of selenium, omega-6, arachidonic acid, omega-3 and EPA and 95% confidence intervals (dotted lines) displayed separately for women and men. All models were adjusted for age, energy, BMI and education. The x-axis was truncated showing 5 to 95 percentile of the intake.

I

Original Paper

Two New Meal- and Web-Based Interactive Food Frequency Questionnaires: Validation of Energy and Macronutrient Intake

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Abstract

Background: Meal-Q and its shorter version, MiniMeal-Q, are 2 new Web-based food frequency questionnaires. Their meal-based and interactive format was designed to promote ease of use and to minimize answering time, desirable improvements in large epidemiological studies.

Objective: We evaluated the validity of energy and macronutrient intake assessed with Meal-Q and MiniMeal-Q as well as the reproducibility of Meal-Q.

Methods: Healthy volunteers aged 20-63 years recruited from Stockholm County filled out the 174-item Meal-Q. The questionnaire was compared to 7-day weighed food records (WFR; n=163), for energy and macronutrient intake, and to doubly labeled water (DLW; n=39), for total energy expenditure. In addition, the 126-item MiniMeal-Q was evaluated in a simulated validation using truncated Meal-Q data. We also assessed the answering time and ease of use of both questionnaires.

Results: Bland-Altman plots showed a varying bias within the intake range for all validity comparisons. Cross-classification of quartiles placed 70%-86% in the same/adjacent quartile with WFR and 77% with DLW. Deattenuated and energy-adjusted Pearson correlation coefficients with the WFR ranged from r=0.33-0.74 for macronutrients and was r=0.18 for energy. Correlations with DLW were r=0.42 for Meal-Q and r=0.38 for MiniMeal-Q. Intraclass correlations for Meal-Q ranged from r=0.57-0.90. Median answering time was 17 minutes for Meal-Q and 7 minutes for MiniMeal-Q, and participants rated both questionnaires as easy to use.

Conclusions: Meal-Q and MiniMeal-Q are easy to use and have short answering times. The ranking agreement is good for most of the nutrients for both questionnaires and Meal-Q shows fair reproducibility.

(J Med Internet Res 2013;15(6):e109) doi:10.2196/jmir.2458

KEYWORDS

validity; reproducibility; food frequency questionnaire; Internet; weighed food record; doubly labeled water; adult

Introduction

The food frequency questionnaire (FFQ) is a commonly used method for assessing diet in large-scale epidemiological studies. The advantages of the FFQ include a low participant burden compared to dietary records, and low cost because it is typically a self-administered method. However, there is a need for methodological improvement, including the FFQ layout and its ease of use.

Most FFQs list food items according to food groups (vegetables, meats, dairy, etc), yet people typically consume food grouped into meals. Moreover, meal-based questionnaire designs have been shown to facilitate recall of dietary intake in previous studies [1,2]. Therefore, we developed a meal- and Web-based FFQ, called Meal-Q, with a design that allows for individually adapted follow-up questions. Thus, participants only answer questions relevant to their own food habits. For example, a high consumer of bread and cheese will get follow-up questions about the number of slices of bread and cheese, whereas a low consumer will not. This feature reduces the answering time and improves the ease of use.

Approximately 90% of the Swedish adult population used the Internet in 2011 [3], justifying development of Web-based questionnaires for national population-based studies. Furthermore, the Web-based design makes the use of Meal-Q more cost-efficient than a paper-based FFQ and facilitates assessment of large samples. The ability to use built-in checks for missing answers and the immediate transfer of answers into digital format also assures complete data collection and improves data quality [4,5].

We evaluated the validity and reproducibility of energy and macronutrient intake assessed with Meal-Q by comparing it to a weighed food record (WFR) and doubly labeled water (DLW). By using truncated data from Meal-Q, we also validated a shorter version called MiniMeal-Q.

Methods

Background

The development of Meal-Q was based on results from a population-based study in which 700 randomly selected Swedish participants reported, through either face-to-face interviews or telephone 24-hour recalls, on the food products they consumed for breakfast, lunch, dinner, and snacks (E Möller and S Christensen, personal written communication, August 2008). This dietary information guided the design of a meal- and Web-based FFQ called MaxMeal-Q. After a pretest of MaxMeal-Q in a randomly selected group of individuals (N=216), the shorter version, Meal-Q, was formed by omitting less commonly consumed food items and dishes. Subsequently, Meal-Q was included in the Validation of Methods Assessing diet and physical activity (VALMA) study. The reference methods were a 7-day WFR on the Web and DLW for estimation of energy expenditure [6].The Research Ethics committee at Karolinska Institutet approved the study.

After the validation study was completed, researchers from LifeGene, a large population-based cohort study [7], decided to use Meal-Q under the condition that the answering time be reduced. Therefore, we developed the shorter version, MiniMeal-Q, by omitting food items consumed on average with a low intake frequency and that contributed least to the total energy and nutrient intake. Yet, food items representing important food sources of certain nutrients were kept (eg, black pudding that contributes to iron intake). After a time test, LifeGene decided to use MiniMeal-Q. We validated MiniMeal-Q in the present study by truncating Meal-Q data to simulate MiniMeal-Q. The inherent dependence between Meal-Q and MiniMeal-Q should be taken into account when comparing their validity.

Recruitment

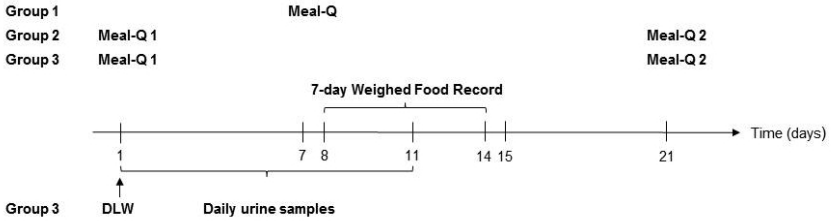
In April 2009, 180 healthy volunteer men and women aged 20 to 63 years were recruited to the VALMA study through public advertisement in Stockholm County, Sweden. Recruitment also took place at 3 universities including announcements among nutritionist students. Access to the Internet and an email address were prerequisites for eligibility, as well as not being on a weight-loss diet, not being pregnant, and not having given birth within the past 10 months. At an introductory meeting, participants were informed about the study and signed informed consent forms. Participants self-reported their height and weight, which was used to calculate body mass index (BMI).

Study Design

After recruitment, the participants were divided into 3 age- and gender-balanced groups: group 1 (n=87), group 2 (n=53), and group 3 (n=40). Each group followed a 3-week study scheme shown in Figure 1.

All groups filled out Meal-Q and the WFR on the Web at their own choice of location (eg, at home) and group 3 was also given DLW. Groups 2 and 3 filled out a second Meal-Q after 3 weeks. Data from the first administered Meal-Q assessment and the WFR from all groups were compared for validity evaluation. The data from the first Meal-Q assessment was truncated for simulated analysis of MiniMeal-Q. The first and second Meal-Q assessments from groups 2 and 3 were compared for reproducibility evaluation. Information about education, occupation, and tobacco use (smoking and Swedish snuff) was collected in the first questionnaire. Answering time was automatically recorded, and directly after completion of the first Meal-Q, a short Web survey followed to evaluate its ease of use.

Figure 1. The 3-week study scheme of the VALMA study.



Dietary Assessment

Meal-Q

The interactive Meal-Q included 102 to 174 food items (depending on the number of follow-up questions) and asked about dietary intake during the past few months. For an example of a questionnaire module, see Figure 2. Meal-Q assessed intake of (1) food items, dishes, and beverages, (2) energy and nutrients, including alcohol, (3) supplements, (4) meal patterns, and (5) eating behavior, such as restaurant visits, intake of fast food, light products, probiotics, and the use of cooking fat and salt. Respondents chose from predefined food items and intake frequencies and only filled in what they ate at least once a month. For each of the following food groups, 5 photos of portion sizes were included: (1) rice, potatoes, and pasta, (2) meat, chicken, fish, and vegetarian substitutes, and (3) vegetables (raw or cooked). The photos were used to calculate portion sizes for cooked dishes and vegetables. For other food items, standard portion sizes were used based on information from the National Food Agency, the Swedish Consumer Agency, measured portion sizes developed by the research group, as well as standard portion sizes used in other FFQs at Karolinska Institutet.

MiniMeal-Q

MiniMeal-Q includes 75 to 126 food items—approximately 30% fewer items than Meal-Q—and has similar questions on meal patterns and eating behavior. After the VALMA study was finished, MiniMeal-Q was sent out to 79 volunteer VALMA participants to assess answering time and ease of use.

Weighed Food Records on the Web

At study start, participants were given oral instructions, a kitchen scale, and a handbook with instructions on how to complete the 7-day WFR by using a Web-based program. Participants were asked to weigh and report all consumed food products and beverages at the highest detail level possible (eg, each food item in a dish was encouraged to be reported in its individual weight). The participants could choose among over 2000 food items in the program's food database, and they also recorded which day they consumed the food item as well as for which meal (ie, breakfast, lunch, dinner, or between meals). Data collectors checked all records for completeness. In the program, participants also provided a 7-day pedometer-based physical activity record. The participants were asked to report their total number of daily steps as well as other activities not captured by pedometers, such as bicycling or swimming. From this, the physical activity level (PAL) was calculated for each participant and the information was used for identification of potential energy underreporters in the WFR by using the Goldberg cut-off method [8].

Nutrient Database

Intake of food items and dishes from Meal-Q, MiniMeal-Q, and the WFR were converted into energy (kJ/day) and macronutrient (g/day) intake using the national database on nutrient content published by the Swedish National Food Agency [9]. The nutrient conversion for the questionnaires was done by computer programs developed and validated specifically for this study, whereas the conversion of the WFR was done with the Web-based WFR program. Dietary supplements were not included in the analyses.

Doubly Labeled Water Method

Total energy expenditure was determined in group 3 (n=40) using the DLW method [10] over 11 consecutive days (Figure 1). The details of this procedure have been described previously [11]. Briefly, on day 1 at the study site, each participant provided a 5-milliliter urine sample before receiving an oral DLW dose calculated according to body weight [12]. Subsequently, daily spot urine samples were collected for a total of 9 days. Participants were instructed to collect urine samples at a similar time each day (but not the first void of the day). All samples were kept refrigerated. On day 11, the 9 urine samples were returned to the study site and the eleventh urine sample was collected. All samples were shipped to the Medical Research Council, Human Nutrition Research, Cambridge, United Kingdom, for isotopic analysis, which has been previously described in detail [13]. Enrichments of $^{2}H/^{1}H$ and $^{18}O/^{16}O$ in urine samples were determined by mass spectrometry. Following conversion to the universally accepted Vienna Standard Mean Ocean Water (VSMOW) / Standard Light Arctic Precipitation (SLAP) scale, total energy expenditure (TEE) was calculated by using standard equations [14-16]. CO_2 production (mole/day)

was estimated using Schoeller et al's correction for fractionation [15] and a respiratory quotient of 0.85. The respiratory quotient is based on omnivores with 30% to 35% energy contribution from fat and suitable to the VALMA population. The results of the CO_2 production were used to calculate the TEE of each participant by using the modified Weir equation [17].

Statistical Analysis

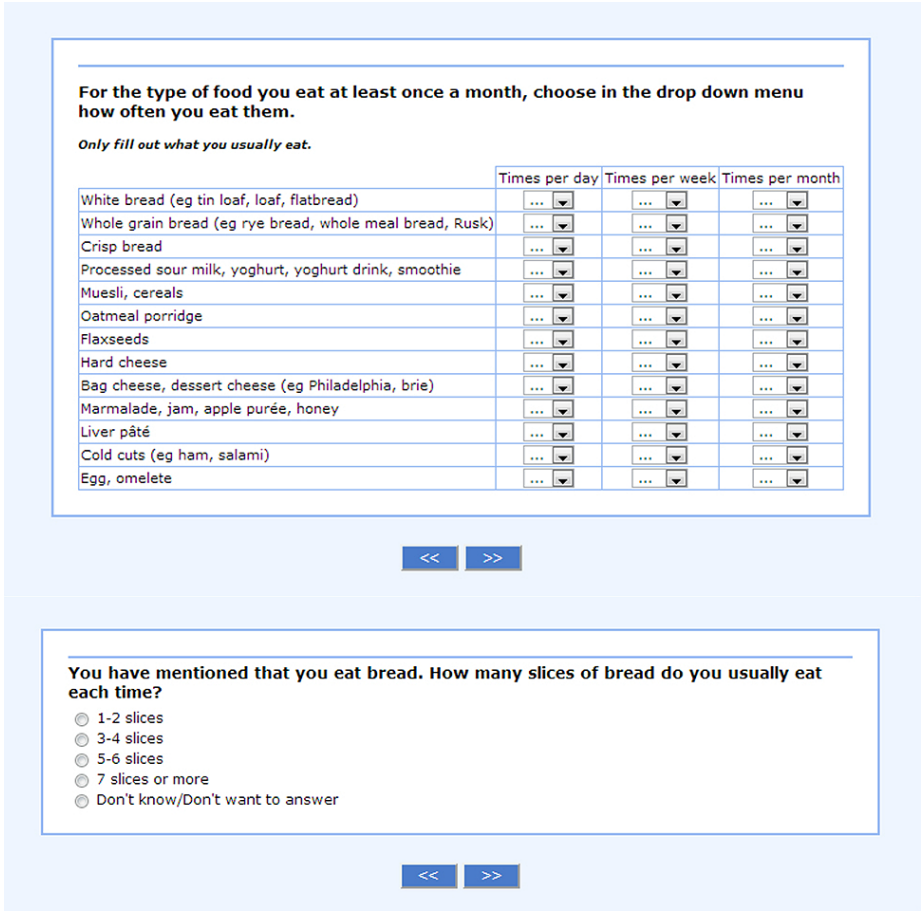
Descriptive characteristics of study participants are presented as mean (SD) and as counts (%). Differences in BMI and age between study groups, between men and women, and between included and excluded participants were assessed using a 2-sample *t* test. Differences in education, nutrition background (studying or working in the nutrition field), and tobacco use were assessed using Fisher's exact test. The level of statistical significance was set to alpha =.05.

Median and interquartile range (IQR) of crude energy and macronutrient intake was calculated and compared among Meal-Q, MiniMeal-Q, and the WFR. Energy intake from the questionnaires was also compared to TEE from DLW. Wilcoxon signed rank tests were used to determine differences between all methods. The median (IQR) answering time in minutes of each questionnaire was calculated and ease of use was evaluated from the Web survey. The between-person variance captured in the truncated MiniMeal-Q as compared to Meal-Q was calculated using linear regression.

For validity and reproducibility analyses, macronutrients were adjusted for total energy intake using the residual method [18]. Variables deviating from the normal distribution were transformed using the square, square root, or log transformation, as appropriate. Absolute agreement and potential difference in bias within the intake range were evaluated by plotting the differences between questionnaires and WFR or DLW against the average of the 2 methods, according to the method of Bland and Altman [19]. The degree of variation was represented by the limits of agreement, ie, ±2 SD of the mean difference. The ranking agreement and magnitude of misclassification when comparing questionnaires with the WFR and DLW was tested by dividing participants into quartile categories of energy and energy-adjusted macronutrient intake. Proportions of participants classified into the same, adjacent, and extreme quartiles were calculated. Because variables were normally distributed after energy adjustment and transformation, Pearson correlation coefficients were used to measure the degree of linear relationship between the questionnaires and the WFR and DLW. Deattenuated correlations corrected for within-person variation in the WFR were calculated using the formulas of Beaton et al [20] and Liu et al [21], and confidence intervals (CI) were produced using the method of Willett and Rosner [22]. Confidence intervals for correlations with DLW were obtained using the bootstrap method [23].

Reproducibility of Meal-Q was evaluated by comparing crude median energy and macronutrient intake between the first and second Meal-Q and by cross-classification of energy and energy-adjusted [18] quartiles of macronutrient intake. Intraclass correlation coefficients (ICCs) [24] were also computed using 1-way ANOVA with random effects. Statistical analyses were performed using STATA statistical software version 11.2 (StataCorp LP, College Station, TX, USA).

Figure 2. Screenshot of a Meal-Q module: breakfast and snack items and follow-up question on bread (translated from the Swedish questionnaire version in the VALMA study).



Results

One participant was excluded due to dropout (group 1) and 2 others due to illness (group 2 and 3). Eleven participants (4 in group 1, 6 in group 2, and 1 in group 3) were identified as energy underreporters by applying the Goldberg cut-off [8] on energy intake from the WFR together with data from each participant's calculated PAL. Additionally, 3 underreporters (group 3) in the WFR were identified using individual PAL values calculated from DLW data. Because of the implausible energy intake by the WFR, the 14 underreporters were excluded for the validity comparison between Meal-Q, MiniMeal-Q, and the WFR; therefore, 163 participants remained (group 1: n=82; group 2: n=46; and group 3: n=35). For the validity comparison between Meal-Q, MiniMeal-Q, and DLW in group 3, no exclusion of energy underreporters was made; therefore, 39 participants remained. For the reproducibility analysis of Meal-Q, 4 participants had missing values in the second administered Meal-Q; therefore, 87 participants remained. We found no statistically significant differences between included and excluded participants in terms of age, BMI, education, nutrition background, or tobacco use.

Descriptive Statistics

As shown in Table 1, most of the study participants were students or highly educated. One-third were working full time, and almost as many had a nutrition background. Few participants used tobacco. There was no statistically significant difference between groups or sexes regarding age, BMI, education, nutrition background, or smoking (but more men than women used Swedish snuff).

Table 1. Characteristics of the participants in the validation study (n=167[a]).

Characteristics	By group			By gender		All (n=167)
	Group 1 (n=82)	Group 2 (n=46)	Group 3 (n=39)	Men (n=35)	Women (n=132)	
Gender, n (%)						
Male	16 (19.5)	11 (23.9)	8 (20.5)			35 (21.0)
Female	66 (80.5)	35 (76.1)	31 (79.5)			132 (79.0)
Age (years), mean (SD)	34 (12)	31 (11)	33 (12)	33 (10)	33 (12)	33 (12)
BMI (kg/m^2), mean (SD)	23 (3.6)	23 (3.4)	23 (3.7)	24 (2.2)	23 (3.8)	23 (3.6)
Education >12 years, n (%)	64 (78.0)	38 (82.6)	32 (82.1)	28 (80.0)	106 (80.3)	134 (80.2)
Working full time, n (%)	33 (40.2)	12 (26.1)	10 (25.6)	12 (34.3)	43 (32.6)	55 (32.9)
Student, n (%)	41 (50.0)	31 (67.4)	26 (66.7)	19 (54.3)	79 (59.8)	98 (58.7)
Background in nutrition[b], n (%)	21 (25.6)	15 (32.6)	13 (33.3)	6 (17.1)	43 (32.6)	49 (29.3)
Tobacco use[c], n (%)	11 (13.4)	5 (10.9)	6 (15.4)	12 (34.3)	10 (7.6)	22 (13.2)

[a]From this study sample, 4 underreporters were excluded for analysis with the WFR (n=163). There were no statistically significant differences in characteristics between groups or sexes, except for Swedish snuff between sexes (1.8% women and 4.2% men, *P*=.001) via 2-sample *t* test and Fisher's exact test.

[b]Studying or working in the nutrition field.

[c]Tobacco use = smoking and/or Swedish snuff. Values are missing for 3 women in group 3.

The median time to answer the Meal-Q and the MiniMeal-Q was 17 (IQR 11) and 7 (IQR 4) minutes, respectively. Most (92%) participants perceived Meal-Q as easy to fill out, 91% thought the questions were relevant, and 93% reported that food items and dishes were presented in a logical order. For MiniMeal-Q, the figures were 95%, 88%, and 91%, respectively. The overall mean grade of Meal-Q and MiniMeal-Q's ease of use was 4.2 on a 5-point scale in which 5 was the best grade. The between-person variance captured by MiniMeal-Q as compared to Meal-Q ranged from 96% to 99% for energy and macronutrients.

Validity

Energy and macronutrient intake was higher in the WFR compared with both questionnaires, except for polyunsaturated fat assessed with Meal-Q (Table 2). In group 3 (n=39), the energy expenditure from DLW was higher than energy intake assessed by both questionnaires (*P*<.001, Wilcoxon's signed rank test). The energy expenditure from DLW was 11,423 kJ (IQR 2777) and the energy intake assessed with Meal-Q and MiniMeal-Q were 7954 kJ (IQR 2736) and 7358 kJ (IQR 2718), respectively.

As shown in Figure 3, the Bland-Altman plots with DLW indicate that the WFR and both questionnaires underestimated energy intake for most participants. Compared to the WFR, the questionnaires had a larger underestimation, larger variance, and a weak trend of decreasing accuracy with increasing intakes.

The Bland-Altman plots of Meal-Q and the WFR in Figure 4 showed a negative mean difference for energy and all macronutrients. There was a trend of decreasing accuracy with increasing energy and polyunsaturated fat intake, and trends of increasing underestimation with increasing intakes for other macronutrients. Because of varying bias within the intake range, the proportion of participants outside the limits of agreement deviated somewhat from 5% for some plots. Bland-Altman plots for MiniMeal-Q and the WFR were very similar to those for Meal-Q and the WFR (see Multimedia Appendix 1).

Table 3 shows that the proportion of participants classified into the same or adjacent quartile for energy was 70% by Meal-Q and 67% by MiniMeal-Q as compared to the WFR. Correspondingly, the proportions for macronutrients ranged from 76% to 86% for both questionnaires. Quartile cross-classification of Meal-Q and DLW placed 77% into the same or adjacent quartile, and values were identical for MiniMeal-Q.

Pearson correlation coefficients (*r*) with the WFR and DLW were similar between Meal-Q and MiniMeal-Q (Table 4). Deattenuated and energy-adjusted correlations with the WFR ranged from *r*=0.18-0.73 for Meal-Q and from *r*=0.18-0.74 for MiniMeal-Q. Correlation with DLW was *r*=0.42 for Meal-Q and *r*=0.38 for MiniMeal-Q.

Reproducibility

Table 5 shows that there were no statistically significant differences in crude intakes between the first and second Meal-Q assessments. The proportion of participants classified into the same or adjacent quartile ranged from 85% to 96%. ICCs ranged from *r*=0.43-0.92 for crude intakes and from *r*=0.57-0.90 for energy-adjusted macronutrients.

Table 2. Daily crude energy and macronutrient intake assessed with the WFR, Meal-Q, and MiniMeal-Q (n=163).

Energy and macronutrients	WFR	Meal-Q[a]		MiniMeal-Q[a]	
	Median (IQR)	Median (IQR)	% of WFR	Median (IQR)	% of WFR
Energy (kJ)	9183 (2340)	7667 (3723)	83	7017 (3632)	76
Protein (g)	85 (37)	79 (40)	93	70 (34)	82
Carbohydrates (g)	243 (97)	211 (132)	87	190 (124)	78
Total fat (g)	86 (37)	65 (34)	76	62 (35)	72
Saturated fat (g)	33 (18)	22 (14)	67	20 (13)	61
Monounsaturated fat (g)	31 (16)	23 (13)	74	22 (11)	71
Polyunsaturated fat (g)	14 (8)	13 (8)	93	12 (9)	86
Alcohol (g)	6 (15)	5 (8)	85	5 (8)	83

[a]Intakes assessed with Meal-Q and MiniMeal-Q were statistically significantly different from the WFR (P=.01), except for polyunsaturated fat assessed with Meal-Q (P=.28). Intakes assessed with Meal-Q and MiniMeal-Q were statistically significantly different from each other (P<.001) via Wilcoxon signed rank test.

Table 3. Quartile cross-classification of mean daily energy and energy-adjusted[a] macronutrient intake assessed with Meal-Q, MiniMeal-Q, and the WFR (n=136) and cross-classification of mean daily energy intake and energy expenditure measured with DLW (n=39).

Energy and macronutrients	Same quartile, %		Adjacent quartile, %		Extreme quartile, %	
	Meal-Q	MiniMeal-Q	Meal-Q	MiniMeal-Q	Meal-Q	MiniMeal-Q
Energy	26	27	44	40	8.5	7.4
Protein	36	40	40	36	6.7	5.5
Carbohydrate	42	42	40	37	2.5	1.8
Total fat	37	33	41	46	8.0	9.2
Saturated fat	52	45	33	37	4.9	4.3
Monounsaturated fat	44	44	33	33	6.1	6.7
Polyunsaturated fat	33	31	47	49	5.5	4.9
Alcohol	50	49	36	37	4.3	3.7
DLW, energy (kJ)	33	33	44	44	2.6	2.6

[a]Adjustments for total energy intake were made using the residual method [18].

Table 4. Pearson correlation coefficients between Meal-Q, MiniMeal-Q, and the WFR (n=163) and DLW (n=39).

Energy and macronutrients	Crude[a]		Energy-adjusted[a,b]		Deattenuated (95% CI)[c]	
	Meal-Q	MiniMeal-Q	Meal-Q	MiniMeal-Q	Meal-Q	MiniMeal-Q
Energy	0.16	0.16	—	—	0.18 (0.01-0.36)	0.18 (0.01-0.33)
Protein	0.22	0.21	0.30	0.31	0.33 (0.17-0.47)	0.34 (0.18-0.48)
Carbohydrates	0.54	0.54	0.62	0.57	0.65 (0.54-0.74)	0.60 (0.48-0.70)
Total fat	0.06	0.02	0.55	0.49	0.57 (0.45-0.67)	0.51 (0.37-0.62)
Saturated fat	0.15	0.11	0.57	0.54	0.60 (0.48-0.70)	0.57 (0.44-0.67)
Monounsaturated fat	0.13	0.08	0.52	0.46	0.56 (0.43-0.67)	0.50 (0.36-0.62)
Polyunsaturated fat	0.23	0.21	0.36	0.35	0.42 (0.25-0.56)	0.40 (0.23-0.54)
Alcohol	0.64	0.65	0.61	0.63	0.73 (0.59-0.82)	0.74 (0.60-0.83)
DLW, energy (CI)[d]	0.42 (0.16-0.68)	0.38 (0.10-0.66)	—	—	—	—

[a]All correlation coefficients were statistically significant (P = <.001-.046), except for crude total, saturated and monounsaturated fat for both questionnaires (P=.06-.84).

[b]Adjustments for energy were made using the residual method [18].

[c]Deattenuated values were obtained using the formulas suggested by Beaton et al [20] and Liu et al [21]. Confidence intervals were produced using the method suggested by Willett and Rosner [22].

[d]Confidence intervals were obtained using the bootstrap method [23].

Table 5. Daily energy and macronutrient intake assessed with the 2 Meal-Q assessments in groups 2 and 3, quartile cross-classifications and crude and energy-adjusted[a] intraclass correlation coefficients[b] (ICC) (n=87)[c].

Energy and macronutrients	Median (IQR) intake			Quartile cross-classifications, %			ICC (95% CI)	
	Meal-Q 1	Meal-Q 2[c]	Difference[d]	Same	Adjacent	Extreme	Crude	Energy-adjusted
Energy (kJ)	7720 (3567)	7383 (3205)	–125 (2497)	51	34	6.9	0.57 (0.42-0.71)	—
Protein (g)	79 (36)	78 (29)	–1.2 (24)	53	40	2.3	0.52 (0.37-0.67)	0.73 (0.63-0.83)
Carbohydrates (g)	209 (122)	206 (113)	0.7 (82)	52	41	2.3	0.64 (0.51-0.76)	0.67 (0.56-0.80)
Total fat (g)	62 (30)	62 (29)	–1.9 (23)	59	26	5.7	0.47 (0.30-0.63)	0.57 (0.43-0.71)
Saturated fat (g)	20 (11)	21 (13)	–0.9 (7.5)	61	25	3.4	0.43 (0.26-0.60)	0.58 (0.44-0.72)
Monounsaturated fat (g)	22 (12)	23 (10)	–0.4 (8.6)	56	32	3.4	0.50 (0.34-0.66)	0.60 (0.46-0.73)
Polyunsaturated fat (g)	13 (9.0)	13 (8.2)	–0.01 (4.84)	57	36	3.4	0.65 (0.53-0.77)	0.68 (0.56-0.79)
Alcohol (g)	4.6 (8.5)	4.3 (7.0)	–1.0 (2.0)	74	22	1.1	0.92 (0.89-0.95)	0.90 (0.87-0.94)

[a]Adjustments for energy were made using the residual method [18].

[b]Intraclass correlation coefficients [24] were computed using 1-way ANOVA with random effects.

[c]Missing values on Meal-Q 2 for 4 participants.

[d]Meal-Q 1–Meal-Q 2; P=.27-.96 via Wilcoxon signed rank test.

Figure 3. Bland-Altman plots showing the differences in energy intake assessed with the WFR, Meal-Q, and MiniMeal-Q and the energy expenditure measured with DLW plotted against the mean of the 2 methods (n=39). Each data point represents 1 participant. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD).

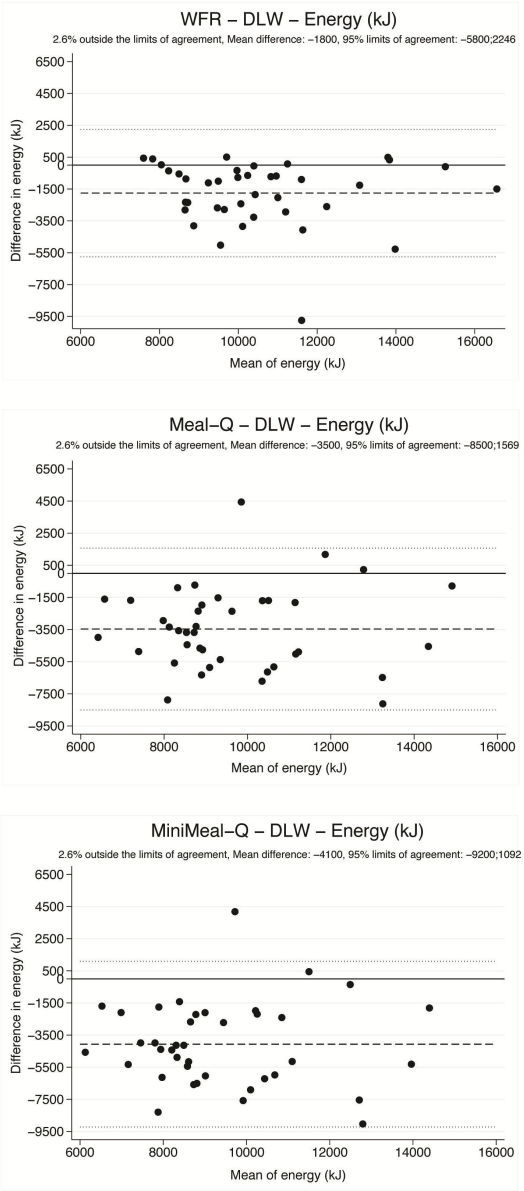
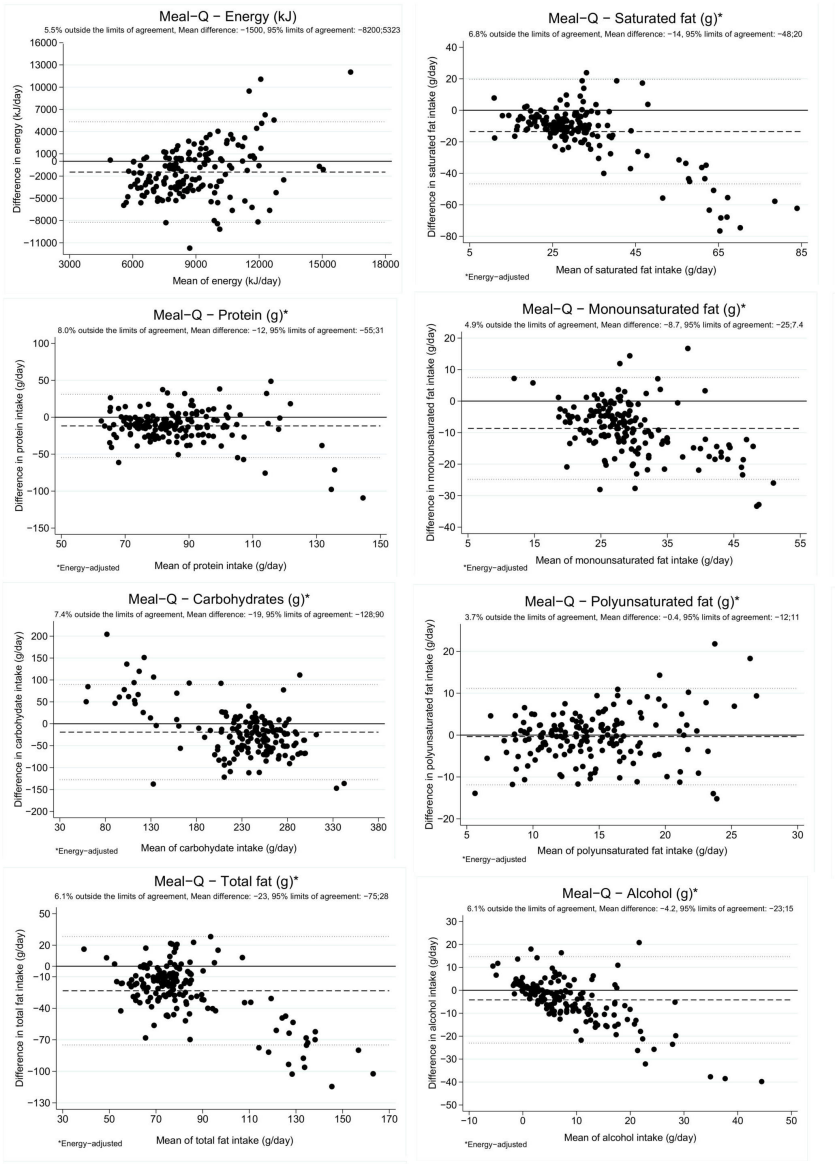


Figure 4. Bland-Altman plots showing the differences in energy, protein, carbohydrate, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, and alcohol intake assessed with Meal-Q and intake assessed with the WFR plotted against the mean of the 2 methods (N=163). Macronutrients are energy-adjusted using the residual method [18].



Discussion

Principal Results

This study presents the validity and reproducibility of the new meal- and Web-based interactive Meal-Q, as well as a simulated validation of its shorter version, MiniMeal-Q. Both questionnaires were perceived as easy to use and had a short answering time. Trends of varying bias within the intake range were seen for energy and all macronutrients. Both questionnaires showed good ranking ability for carbohydrates, total fat, saturated fat, monounsaturated fat, and alcohol, whereas energy, protein, and polyunsaturated fat performed less well. Furthermore, Meal-Q showed fair reproducibility.

Comparison With Prior Work

The Bland-Altman plots of the questionnaires versus WFR and DLW and the plot on WFR versus DLW showed a varying bias within the intake range. Energy and polyunsaturated fat both seemed to be underestimated and overestimated for both questionnaires at higher intake levels. For other macronutrients, the plots indicated that the questionnaires had difficulty assessing higher intakes. In contrast, quartile cross-classification with the WFR showed a fair ranking agreement for most of the nutrients, although a lower agreement was seen for energy and polyunsaturated fat. Similar rankings have been seen in 3 other validation studies of FFQs against food records [25-27] including a Web-based method [25]. In nutritional epidemiology, the association between diet and disease is commonly studied by ranking the dietary intake; therefore, absolute intake is often less important than good ranking order [18]. Hence, despite an underestimation of absolute intake, the ranking agreement for Meal-Q and MiniMeal-Q suggests they are useful in epidemiologic studies regarding most nutrients.

The correlations between the questionnaires and the WFR ranged from 0.18 for energy to 0.74 for alcohol. High correlations between FFQs and diet records are in the order of 0.6-0.7 and it is unlikely that correlations above 0.8 can be obtained [28]. A review of FFQs concluded that the mean correlation with food records of ≥6 days was 0.42 for energy, 0.57 for total fat, 0.53 for protein, 0.58 for carbohydrates, and 0.76 for alcohol [29]. In light of this literature, energy and protein seemed to perform less well, whereas other macronutrients showed correlations within expected ranges. A limitation of the FFQ methodology is the predefined number of food items, frequencies, and portion sizes, which could lead to a "flattened slope" effect in scatter plots [30]. This is a result of respondents consuming little food to unintentionally overreport, and for those consuming a lot to underreport. Correlations from such data would be artificially low. However, a truly small between-person variance would also give similar results [31]. Therefore, the low to moderate correlations seen in this study could reflect a limitation of the questionnaire design, but may also reveal a true small between-person variance. Bland and Altman have discouraged the use of correlation coefficients to evaluate validity because they do not measure agreement [19]. However, because the use of correlations in validation studies is widespread, we have included them to enable comparisons with other studies.

The DLW measurements in group 3 showed that the WFR, Meal-Q, and MiniMeal-Q underestimated energy intake by 17%, 30%, and 36%, respectively. Similar figures for food records and FFQs have been seen in other studies using DLW [32-34]. The Bland-Altman plots showed that the underestimation of energy was considerable for both questionnaires and the large variance indicated difficulties in precision. The underestimation and variance was much smaller for the WFR. Correlations with DLW were moderate for both questionnaires, although the CIs were wide because of the large variance. The correlations were similar to a study by Andersen et al [35], but slightly lower than that of Kroke et al [36]. Despite the underestimation and the large variance, quartile cross-classification with DLW showed a fair ranking agreement, similar to that found by Kroke et al [36].

The moderate to strong quartile cross-classifications of the first and second Meal-Q assessments suggest the questionnaire has fair reproducibility. Correlations between repeated administrations of FFQs in other studies have ranged r=0.5-0.8 [31], and Meal-Q showed similar results. The reproducibility might have been affected by the short time period between the Meal-Q assessments, because participants are less likely to have true changes in intake or response after a short compared to a longer period [37]. In addition, it is important to keep in mind that the reproducibility cannot reveal systematic errors, which can be masked in a high correlation between 2 questionnaires.

The high between-person variance captured by MiniMeal-Q as compared to Meal-Q indicates that it is possible to use a shorter questionnaire while still assessing a similar intake range and keeping the ranking ability. Because MiniMeal-Q originates from Meal-Q data and is also compared to the same reference methods, their results become highly related. Therefore, caution must be taken when comparing their assessments and relative validity.

Regular use of the Internet in Sweden is higher among young people compared to older people. Among those aged 16 to 44 years, 88% to 94% use the Internet daily, whereas the proportion among the age groups 45 to 54 years and 65 to 74 years are 82% to 83% and 38% to 49%, respectively [3]. However, access to the Internet is high for all age groups—more than 90% for the young and middle-aged and 67% to 78% for the oldest age group. It is worth noticing that problems with cognition might be an issue in very old age groups, although this would also hold true for dietary assessment using a paper-based questionnaire. Concerns could be raised regarding whether Web-based questionnaires produce different kinds of bias as compared to paper-based questionnaires. However, bias associated with Web-based data collection does not seem to differ from that of paper-based questionnaires as seen in a large Swedish feasibility study of more than 45,000 participants [38].

Limitations and Strengths

To estimate the validity of a dietary assessment method, 2 statistical assumptions should be fulfilled. First, the assessed dietary intake should be linearly related to true intake. Second, the measurement errors should be independent between the test and the reference method. In this validation study, variables were linearly correlated to the WFR, although energy, protein,

and polyunsaturated fat had a weaker linear relationship. The questionnaires rely on memory and have predefined food items, frequencies, and portion sizes, whereas the WFR does not rely on memory, is open-ended, and has direct assessment of portion sizes. Nevertheless, the methods are linked to the same nutrient database and are likewise affected by social desirability, which could lead to an overestimated validity.

The strengths of this validation study include its large sample size and few dropouts. There was also high compliance to the questionnaires, the WFR and DLW. Using the DLW method is an additional strength that enabled an objective estimation of TEE for the evaluation of energy intake. The digital format of the questionnaires and the WFR also substantially reduced the risk of coding errors and missing data. The proportion of underreporters in this study (14/177, 8%) was notably lower compared with some other studies [39-42], even if studies have had proportions in the range of 2% to 85% [43]. The use of individual PAL values from each participant for the Goldberg cut-off is likely to have increased the sensitivity [44] and could be an explanation.

Due to time constraints, the study period had to be kept short. This could have given an overestimation of the validity because the questionnaires and the WFR assessments were performed within a short period of time. Furthermore, the WFR was only performed once. Ideally, several records with independent days spread over a longer time period would have reflected the habitual dietary intake better. However, corrections for within-person variance in the WFR were made to minimize day-to-day variation and energy adjustment was made to avoid variations in intake related to total energy intake. The DLW measurement should also preferably have been done repeatedly over a longer time period to reflect habitual energy intake; however, this was not possible. Furthermore, most of the participants were women and many were students with a nutrition background. Also, the self-selection of participants could have biased the sample in favor of more motivated participants who are more inclined to give accurate answers compared to a sample from the general population [45]. Nevertheless, dietary intake from the WFR was in-line with a Swedish national dietary survey using food records (n=1214) [42], suggesting that the WFR intake would be comparable to an assessment within a more general and less-selected population. Acknowledging the highly educated study sample, the answering time might have been longer in a less-educated population.

Conclusions

Meal-Q and MiniMeal-Q are 2 Web-based FFQs shown to be highly user-friendly. Despite their short answering time, they had an ability to rank most macronutrient intakes well compared with the reference methods. In addition, Meal-Q showed fair reproducibility.

Acknowledgments

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Authors' Contributions

The authors' responsibilities were as follows—SEC, EM, SEB, LL, OB, KB: questionnaire design; SEC, EM, SEB, LL, KB: validation study design; SEC, EM, SEB, KB: data collection; AW: analyses of DLW and interpretation of results; SEC, KB, OB: development and validation of the nutrient calculation programs MealCalc and MiniMealCalc; OB: calculation of nutrients; SEC, AS, AP: statistical analyses; SEC, AP, AS, LL, KB: interpretation of results; SEC: drafted the manuscript; and SEC, EM, SEB, AP, AW, AS, OB, LL, KB: review and revision of the manuscript including approval of final version.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Bland-Altman plots showing the differences in energy, protein, carbohydrate, total fat, saturated fat, monounsaturated fat, polyunsaturated fat and alcohol intake assessed with MiniMeal-Q and intake assessed with the WFR plotted against the mean of the two methods (n=163). Macronutrients are energy-adjusted using the residual method [19].

[JPG File, 1MB - jmir_v15i6e109_app1.jpg]

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Abbreviations

BMI: body mass index
DLW: doubly labeled water
FFQ: food frequency questionnaire
ICC: intraclass correlation coefficient
PAL: physical activity level
SLAP: Standard Light Arctic Precipitation
TEE: total energy expenditure
VSMOW: Vienna Standard Mean Ocean Water
VALMA: validation of methods assessing diet and physical activity
WFR: weighed food record

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Relative validity of micronutrient and fiber intake assessed with two new interactive meal- and web-based food frequency questionnaires

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ABSTRACT

Background: The two meal- and web-based food frequency questionnaires Meal-Q and MiniMeal-Q were developed for cost-efficient assessment of dietary intake in epidemiological studies.

Objective: To evaluate the relative validity of micronutrient and fiber intake assessed with Meal-Q and MiniMeal-Q. Secondly, the reproducibility of Meal-Q was evaluated.

Methods: A total of 163 volunteer men and women aged 20-63 were recruited from Stockholm County, Sweden. Assessment of micronutrient and fiber intake with the 174-item Meal-Q was compared to a web-based 7-day weighed food record (WFR). Two administered Meal-Q questionnaires were compared for reproducibility. The 126-item MiniMeal-Q, developed after the validation study, was evaluated in a simulated validation by using truncated Meal-Q data.

Results: The study population consisted of about 80% women with a mean age of 33 years and was on average highly educated. Cross-classification of quartiles with the WFR placed 69-90% in the same/adjacent quartile for Meal-Q and 67-89% for MiniMeal-Q. Bland-Altman plots with the WFR and the questionnaires showed on average large variances and a trend of increasing underestimation with increasing intakes. De-attenuated and energy-adjusted Spearman correlations between the questionnaires and the WFR were in the range r=0.25-0.69, excluding sodium that was not statistically significant. Cross-classifications of quartiles of the two Meal-Q administrations placed 86-97% in the same/adjacent quartile and intra-class correlations for energy-adjusted intakes were in the range r=0.50-0.76.

Conclusions: With the exception of sodium, this validation study demonstrates Meal-Q and MiniMeal-Q to be useful methods for ranking micronutrient and fiber intake in epidemiological studies with web-based data collection.

Keywords: validity, reproducibility, FFQ, micronutrients, weighed food record, Internet, adult

INTRODUCTION

The increasing use of the Internet worldwide [1] has made web-based food frequency questionnaire (FFQ) methodology an attractive alternative to traditional paper-based instruments in epidemiological research. Today, over 90% of the Swedish adult population has Internet access [2], which is a convincing rationale for choosing the web over the paper-and-pencil method. Compared to paper-based FFQs, expenses are dramatically lower for web-based versions regarding both dissemination and data handling, making it a more cost-efficient method [3,4]. In addition, the costs for the required software infrastructure have decreased over the past years [5]. The dynamic nature of the web enables an interactive design with follow-up questions and skip patterns, adapting the questions to the respondent's answers and thereby reducing the answering time. An interactive web-questionnaire has previously shown high compliance in a Swedish population with widespread Internet access [6]. Taking advantage of the benefits of using the web, we have developed two web-based FFQs with an interactive design; Meal-Q and MiniMeal-Q. The questionnaires have a meal-based format to ease recall of food intake. This approach has shown promising results in previous studies when compared to traditional food group designs [7,8].

We have previously published results on the validity of energy and macronutrient intake assessed by Meal-Q and MiniMeal-Q with doubly labeled water and a weighed food record (WFR) as reference methods [9]. The present paper evaluates the validity of micronutrient and fiber intake assessed by Meal-Q and MiniMeal-Q by using the WFR as reference method. We also present an evaluation of the reproducibility of Meal-Q.

SUBJECTS AND METHODS

Background

Meal-Q was developed with guidance from a population-based cross-sectional study on food products consumed for breakfast, lunch, dinner and snacks meals as reported by 700 randomly selected Swedish subjects through either face-to-face interviews or 24-hour recalls (Personal written communication by E Möller, and S Christensen, August 2008). In the spring of 2009, Meal-Q was evaluated in the validation study VALMA; VALidation of Methods Assessing diet and physical activity. The reference method was a 7-day WFR on the web. After the VALMA study was completed, we developed the shorter version MiniMeal-Q by omitting food items with low consumption frequency and low contribution to total energy and nutrient intake. However, food items making up important sources of specific nutrient intakes were kept, e.g. black pudding, which contributes to iron intake. Moreover, varieties of similar food items were also kept to enable analyses of dietary patterns, e.g. different types of bread and cereals with varying fiber and sugar content. By using truncated data from Meal-Q, we simulated a validity evaluation of MiniMeal-Q. Acknowledging that MiniMeal-Q originates from Meal-Q data, their validity comparison should be interpreted with caution.

Recruitment

A total of 180 healthy volunteer men and women aged 20-63 were recruited through public announcement in Stockholm County, Sweden, to participate in the VALMA study. Announcements were made in the city, the suburbs and at two universities, including amongst others students in nutrition. Prerequisites for eligibility were access to the Internet and an e-mail address, as well as not being on a weight-loss diet, nor being pregnant or having given birth during the last ten months. All subjects were informed about the study at an introductory meeting and gave their written informed consent. The Research Ethics committee at Karolinska Institutet approved the study.

Study design

A study scheme of the 3-week validation study is shown in Figure 1. Subjects were divided into two groups balanced on gender and age: group 1 (n=87) and group 2 (n=93). Group 1 filled out Meal-Q once, while group 2 also filled out a second Meal-Q after three weeks. Validity analysis with the WFR was made using data from each subject's first administered Meal-Q. For the simulated validity analysis of MiniMeal-Q, data from the first Meal-Q from both groups was truncated and compared to the WFR. For reproducibility analysis, the first and second Meal-Q from group 2 were compared. The first administered Meal-Q additionally included questions on education, occupation and tobacco use (current smoking and Swedish snuff use). Each subject self-reported their height and weight, which were used to calculate body mass index (BMI, kg/m^2).

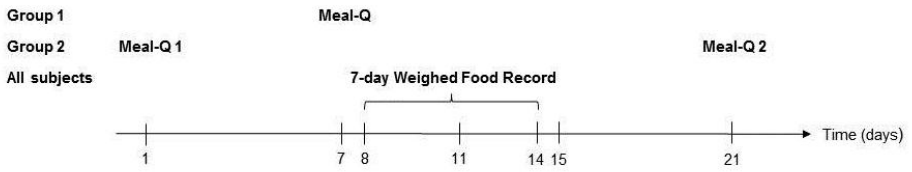

Figure 1. The 3-week study scheme of the VALMA study. Data from the first administered Meal-Q from both groups was compared to the WFR for validity analyses. The same data from Meal-Q was truncated for simulated validity analysis of MiniMeal-Q. Meal-Q was distributed twice in group 2 for reproducibility analysis.

Dietary assessment

Meal-Q

Meal-Q is interactive and includes 102-174 food items depending on the number of follow-up questions (see Figure 2 for a questionnaire module example). It had a mean (SD) answering time of 17 (11) minutes in the current study population [9]. The interactivity implies follow-up questions for high consumers of certain food items and dishes. Meal-Q assesses intake of

food items, dishes and beverages, which enables the calculation of energy and nutrient intake (including alcohol). It also asks about meal patterns, eating behavior such as restaurant visits, intake of fast food, light products, probiotics, the use of cooking fat and salt, as well as the use of dietary supplements. Respondents choose among predefined food items and intake frequencies ranging from 1-3 times a month to 5+ times a day. Five photos of portion sizes are included for each of the following food groups: 1) rice/potatoes/pasta, 2) meat/chicken/fish/vegetarian alternatives and 3) vegetables (raw or cooked). The photos are used to calculate portion sizes for cooked dishes and vegetables, while standard portion sizes are used for other food items. The standard portion sizes are derived from the National Food Agency, the Swedish Consumer Agency, measured portion sizes developed by the research group and standard portion sizes used in other FFQs at Karolinska Institutet. For this validation study, Meal-Q asked about dietary intake during the past few months.

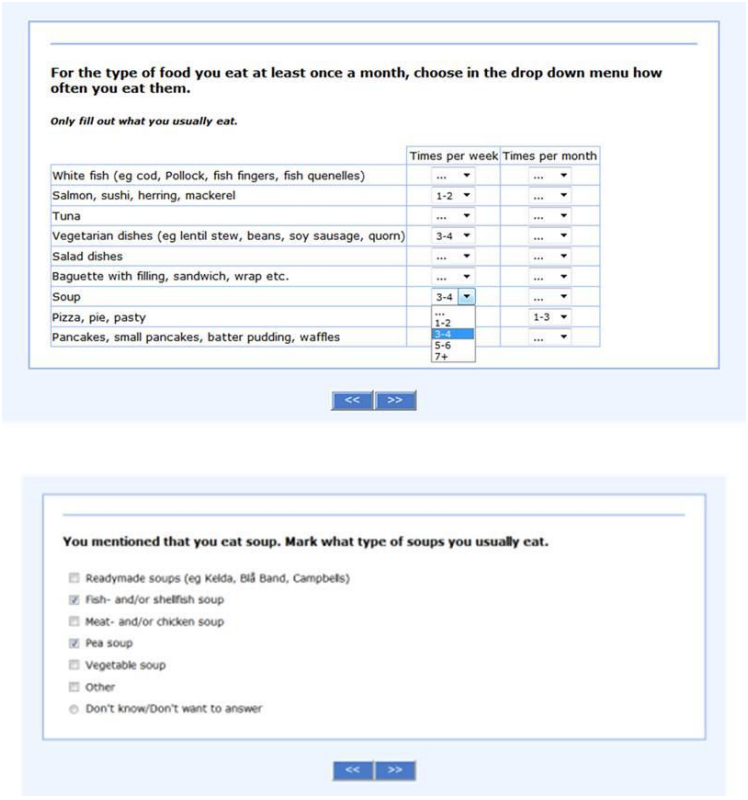

Figure 2. Screen shot of a Meal-Q module: lunch and dinner dishes and a follow-up question on soup. Translated from the Swedish questionnaire version in the VALMA study.

MiniMeal-Q

MiniMeal-Q contains 75-126 food items and is identical to Meal-Q in its design including the interactive feature with adapted follow-up questions. The mean (SD) answering time for MiniMeal-Q has been measured as 7 (4) minutes in a sub-sample of the current study population [9].

7-day Weighed Food Record on the web

At the introductory meeting subjects were given oral instructions and a handbook on how to fill out the 7-day WFR using a web-based program, which covered over 2000 food items. Each subject was given a kitchen scale and was asked to weigh and report all consumed food products and beverages at the highest detailed level possible. For example, a dish was encouraged to be reported by its individually weighed food items. As a help for the recording throughout the day, all subjects were provided paper diaries. All records in the web-based program were checked for completeness and reasonableness by the data collectors.

Nutrient database

Daily intake of micronutrients and fiber was retrieved by linking intake of food items and dishes assessed with Meal-Q, MiniMeal-Q and the WFR to the national database on nutrient content published by the Swedish National Food Agency [10]. The questionnaire's nutrient conversion was made by computer programs (MealCalc and MiniMealCalc) developed and validated by the research group specifically for this study. The nutrient conversion of the food items and dishes assessed with the WFR was built in to the web-based WFR program. The nutrient conversions did not include dietary supplements.

Assessment of physical activity level (PAL) for identification of energy under-reporters

A 7-day pedometer-based physical activity record provided in conjunction with the WFR program was filled out by all subjects. The information was used to calculate the physical activity level (PAL) for each subject. Individual PAL values were also obtained from measurements of energy expenditure by the doubly labeled water (DLW) method [11] on 39 subjects in group 2. A detailed description of the use of the DLW method in the VALMA study has been published previously [9,12]. The PAL values derived from the pedometers and from the DLW measurements were used for identification of potential energy under-reporters in the WFR, in order to exclude them from the comparison with Meal-Q and MiniMeal-Q.

Statistical analysis

Descriptive characteristics of the study subjects are presented as mean (SD) and as counts (%). A two-sample *t*-test was used to assess differences in BMI and age between study groups, between men and women and between included and excluded subjects. Fisher's exact test was used to assess differences in education, nutrition background (studying or working in the nutrition field) and tobacco use. All tests were two-sided with significance level $\alpha = 0.05$.

Median (IQR) crude micronutrient and fiber intake was calculated and compared between Meal-Q, MiniMeal-Q and the WFR, and differences between the methods were determined using Wilcoxon signed rank tests. Linear regression was used to calculate the between-person variance captured in the truncated MiniMeal-Q as compared to Meal-Q. Identification of energy under-reporters was made using the Goldberg cut-off method [13]. The cut-off was calculated using the energy intake from the WFR together with the obtained PAL values from the physical activity record and the doubly labeled water data.

For validity and reproducibility analyses micronutrient and fiber intakes were adjusted for total energy intake using the residual method [14]. To test the ranking agreement and magnitude of misclassification of the questionnaires in comparison to the WFR we used quartile cross-classifications, calculating proportions of subjects classified into the same, adjacent and extreme quartiles of energy-adjusted intakes. Bland-Altman plots were presented for Meal-Q, MiniMeal-Q and the WFR to evaluate absolute agreement and differences in bias within the intake range [15]. The differences between the questionnaires and the WFR were plotted against the mean of the two methods and the degree of variation was represented by the limits of agreement, i.e. ±2 SDs of the mean difference. A majority of the variables were not normally distributed after energy adjustments and therefore Spearman's rank correlation coefficients were used to compare the questionnaires to the WFR. De-attenuated correlations corrected for within-person variation in the WFR were calculated using the formula of Beaton *et al* [16] and Liu *et al* [17] and confidence intervals were produced using the method of Willett and Rosner [18].

Reproducibility of Meal-Q was evaluated by comparing crude median (IQR) micronutrient and fiber intake between the first and second Meal-Q and by cross-classification of energy-adjusted [14] quartiles. Intra-class correlation coefficients (ICCs) [19] were also computed using one-way ANOVA with random effects.

Statistical analyses were performed using STATA statistical software (version 11.2; STATA Corp, College Station, TX).

RESULTS

Exclusions

One subject was excluded due to drop-out (group 1) and two due to illness (group 2). With the Goldberg cut-off method, 14 subjects (four in group 1, ten in group 2) were identified as energy under-reporters in the WFR and were excluded. Hence 163 subjects remained for validity analysis; group 1: n=82; group 2: n=81. We found no significant differences between included and excluded subjects in terms of age, BMI, education, nutrition background or tobacco use (P = .16-1.00). In the WFR assessments one subject had implausibly high intakes of beta-carotene (>30 000 µg/day) and three other subjects had implausibly high intakes of sodium (>9000 mg/day). They were therefore excluded in each respective nutrient analysis. For reproducibility

analysis of Meal-Q no exclusion of energy under-reporters were made, however four subjects had missing values in the second administered Meal-Q, leaving 87 subjects in the analysis.

Descriptive statistics

General characteristics of the study subjects included in the validity analysis are shown in Table 1. Most subjects were highly educated or students, one third were working full time, nearly a third had a nutrition background and few subjects used tobacco. There were no statistically significant differences between study groups or sexes regarding age, BMI, education, nutrition background, smoking or multivitamin/mineral supplement use (P = .05-1.00). However, more men than women used Swedish snuff (P = .001). The between-person variance in micronutrient and fiber intake captured by MiniMeal-Q as compared to Meal-Q was 70-100%.

Table 1 Characteristics[a] of the subjects included in the validity analysis (n = 163).

	Group 1 (n=82) (men:16, women:66)		Group 2 (n=81) (men:18, women:63)		Men (n=34)		Women (n=129)		All (n=163)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Age (years)	34	(12)	32	(11)	33	(10)	33	(12)	33	(12)
BMI (kg/m^2)	23	(4)	23	(4)	24	(2)	23	(4)	23	(4)
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Education >12 years	64	(78)	66	(81)	27	(79)	103	(80)	130	(80)
Working full time	33	(40)	21	(26)	12	(35)	42	(33)	54	(33)
Student	41	(50)	54	(67)	18	(53)	77	(60)	95	(58)
Nutrition background[b]	21	(26)	28	(35)	6	(18)	43	(33)	49	(30)
Tobacco use[c]	11	(13)	10	(12)	12	(35)	9	(7)	21	(13)
Multivitamin/mineral supplement use[d]	18	(22)	14	(17)	8	(24)	24	(19)	32	(20)

[a]There was no statistically significant difference in characteristics between groups or sexes (P = .05-1.00), except for Swedish snuff between sexes (1.8% women and 4.2% men) (P = .001) (two-sample t-test and Fisher's exact test).

[b]Studying or working in the nutrition field.

[c]Tobacco use = current smoking and/or Swedish snuff use. Values are missing for three women in group 2.

[d]Daily or weekly supplement use assessed with Meal-Q.

Validity

The median (IQR) intake of most nutrients was higher when assessed with the WFR than with Meal-Q and MiniMeal-Q (Table 2). Exceptions were beta-carotene intake, which was higher when assessed with Meal-Q, whereas the intake was comparable between the WFR and MiniMeal-Q. There were no differences between the WFR and Meal-Q with regards to thiamine, folate, magnesium and fiber intake. Nor were there any differences between Meal-Q and MiniMeal-Q regarding any of the nutrients.

Table 2 Median (IQR) daily crude micronutrient and fiber intake[a] assessed with the WFR, Meal-Q and MiniMeal-Q
(n = 163).

	WFR		Meal-Q		MiniMeal-Q	
	Median	(IQR)	Median	(IQR)	Median	(IQR)
Beta-carotene (µg)	2632[b]	(2539)[b]	3372	(2905)	3254	(3079)
Thiamine (mg)	1.5	(0.5)	1.4	(0.8)	1.3	(0.8)
Riboflavin (mg)	1.9	(0.7)	1.7	(0.9)	1.5	(0.8)
Niacin (mg)	36	(14)	15	(9)	14	(8)
Vitamin B6 (mg)	2.3	(1.0)	1.8	(1.0)	1.7	(0.9)
Folate (µg)	334	(167)	315	(210)	289	(193)
Vitamin B12 (µg)	5.7	(3.7)	3.8	(2.4)	3.5	(2.3)
Vitamin C (mg)	121	(92)	101	(82)	94	(74)
Vitamin D (µg)	5.6	(4.0)	4.7	(3.3)	4.4	(3.1)
Vitamin E (mg)	11	(5)	10	(5)	9	(5)
Calcium (mg)	1052	(381)	897	(583)	828	(512)
Iron (mg)	13	(6)	13	(7)	11	(6)
Magnesium (mg)	413	(177)	397	(242)	358	(207)
Phosphorus (mg)	1570	(514)	1433	(731)	1305	(677)
Potassium (mg)	3437	(1332)	3130	(1600)	2837	(1477)
Selenium (µg)	45	(21)	44	(24)	36	(22)
Sodium (mg)	3194[c]	(1212)[c]	2448	(1118)	2158	(1015)
Zinc (mg)	12	(4)	11	(5)	10	(5)
Fiber (g)	25	(15)	26	(20)	23	(18)

[a]Most nutrient intakes assessed with the WFR were higher than intakes assessed with Meal-Q and MiniMeal-Q (P = .00-.03). Exceptions were beta-carotene intake that was assessed higher with Meal-Q (P = .03), while similar comparing the WFR and MiniMeal-Q (P = .19). Thiamine, folate, magnesium and fiber intake were similar between the WFR and Meal-Q (P = .16-.92). There was no difference in intakes between Meal-Q and MiniMeal-Q (P < .001). (Wilcoxon's signed-rank test).

[b]n = 162 due to exclusion of one subject with implausibly high intake.

[c]n = 160 due to exclusion of three subjects with implausibly high intakes.

Quartile cross-classifications of micronutrient and fiber intake with the WFR and the questionnaires (Table 3) placed 69-90% of the subjects into the same or adjacent quartile for Meal-Q, with the highest ranking agreement for fiber and the lowest for sodium. For MiniMeal-Q, the ranking agreement ranged from 67% to 89%, also with fiber having the highest and sodium the lowest agreement. Proportions of subjects in the extreme quartile ranged from 1% to 10% for Meal-Q and from 3% to 11% for MiniMeal-Q; the lowest proportions were found for vitamin C, magnesium and fiber, and the highest for sodium.

Table 3 Quartile cross-classifications of mean daily energy-adjusted micronutrient and fiber intake assessed with Meal-Q, MiniMeal-Q and the WFR (n = 163).

	Same quartile		Adjacent quartile		Same/adjacent quartile		Extreme quartile	
	Percentage of subjects (%)							
	Meal-Q	MiniMeal-Q	Meal-Q	MiniMeal-Q	Meal-Q	MiniMeal-Q	Meal-Q	MiniMeal-Q
Beta-carotene (µg)[a]	41	41	40	42	81	83	4	6
Thiamine (mg)	27	31	44	43	71	74	7	5
Riboflavin (mg)	37	36	37	40	74	76	4	4
Niacin (mg)	36	34	45	45	81	79	4	5
Vitamin B6 (mg)	34	31	41	45	75	76	6	6
Folate (µg)	42	40	38	42	80	82	4	4
Vitamin B12 (µg)	44	39	34	33	78	72	5	4
Vitamin C (mg)	39	38	46	45	85	83	2	3
Vitamin D (µg)	36	35	40	40	76	75	7	6
Vitamin E (mg)	40	42	34	33	74	75	4	4
Calcium (mg)	36	35	38	36	74	71	7	9
Iron (mg)	38	37	41	40	79	77	4	5
Magnesium (mg)	42	39	40	44	82	83	3	3
Phosphorus (mg)	33	34	42	43	75	77	7	7
Potassium (mg)	36	37	42	42	79	79	5	6
Selenium (µg)	41	38	37	42	78	80	4	6
Sodium (mg)[b]	33	35	36	32	69	67	10	11
Zinc (mg)	34	33	43	43	77	77	7	7
Fiber (g)	53	55	37	34	90	89	1	3

[a]n = 162 due to exclusion of one subject with implausibly high intake.
[b]n = 160 due to exclusion of three subjects with implausibly high intakes.

The Bland-Altman plots with the WFR were similar for Meal-Q and MiniMeal-Q (Table 4, Figure 3 (showing an example of eight micronutrients) and Appendices 1-4). Niacin was largely under-estimated by approximately 20 mg for both questionnaires. A majority of the nutrients showed increasing under-estimation with increasing intakes and some also had a trend of increasing variance at higher intakes. In contrast, fiber had a larger variance at lower compared to higher intakes. Most of the nutrients had a varying bias over the intake range, i.e. both under- and overestimation of

intake with a magnitude about the same size of the mean intake. However, zinc, magnesium, potassium and phosphorus showed a less varying bias.

Table 4 Overview of results from Bland-Altman plots[a] of Meal-Q and MiniMeal-Q in comparison with the WFR (n = 163).

	Meal-Q		MiniMeal-Q		Meal-Q and MiniMeal-Q
	Mean difference	(±2 SD)	Mean difference	(±2 SD)	Trends[b]
Beta-carotene (µg)[c]	427	(-4100;4985)	285	(-4300;4873)	Increasing variance with increasing intakes
Thiamine (mg)	0.01	(-1.6;16)	-0.1	(-1.8;1.5)	Increasing variance with increasing intakes
Riboflavin (mg)	-0.2	(-1.3;0.8)	-0.3	(-1.3;0.7)	Increasing under-estimation with increasing intakes
Niacin (mg)	-21	(-36;-5)	-22	(-37;-7)	Increasing under-estimation with increasing intakes
Vitamin B6 (mg)	-0.4	(-1.7;0.9)	-0.6	(-1.9;0.7)	Increasing under-estimation and variance with increasing intakes
Folate (µg)	-15	(-245;215)	-50	(-280;180)	Increasing under-estimation with increasing intakes
Vitamin B12 (µg)	-2.0	(-7.6;3.6)	-2.5	(-8.0;3.0)	Increasing under-estimation with increasing intakes
Vitamin C (mg)	-21	(-142;99)	-29	(-151;93)	Increasing under-estimation and variance with increasing intakes
Vitamin D (µg)	-1.3	(-9.4;6.8)	-1.6	(-9.7;6.6)	Increasing under-estimation with increasing intakes
Vitamin E (mg)	-1.4	(-9.8;6.9)	-2.0	(-10.0;6.4)	Increasing under-estimation and variance with increasing intakes
Calcium (mg)	-113	(-803;576)	-183	(-892;526)	Increasing variance with increasing intakes
Iron (mg)	-1.0	(-10.0;8.0)	-2.5	(-12.0;6.7)	Increasing under-estimation and variance with increasing intakes
Magnesium (mg)	-7.5	(-206.0;191.0)	-41	(-244;162)	Increasing under-estimation with increasing intakes

Phosphorus (mg)	-164	(-779;450)	-291	(-904;322)	Increasing under-estimation with increasing intakes
Potassium (mg)	-315	(-1800;1180)	-640	(-2200;878)	Increasing under-estimation with increasing intakes
Selenium (µg)	-4	(-40;32)	-10	(-46;25)	Increasing under-estimation with increasing intakes
Sodium (mg) [d]	-753	(-2700;1238)	-1000	(-3000;922)	Increasing under-estimation and variance with increasing intakes
Zinc (mg)	-1.0	(-6.5;4.4)	-1.9	(-7.3;3.4)	Increasing variance with increasing intakes
Fiber (g)	2.0	(-16.0;20.0)	-1.5	(-20.0;17.0)	Larger variance at lower intakes than at higher intakes

[a]The Bland-Altman plots are shown in Figure 3 and Appendices 1-4.

[b]Trends are similar for Meal-Q and MiniMeal-Q.

[c]n = 162 due to exclusion of one subject with implausibly high intake.

[d]n = 160 due to exclusion of three subjects with implausibly high intakes.

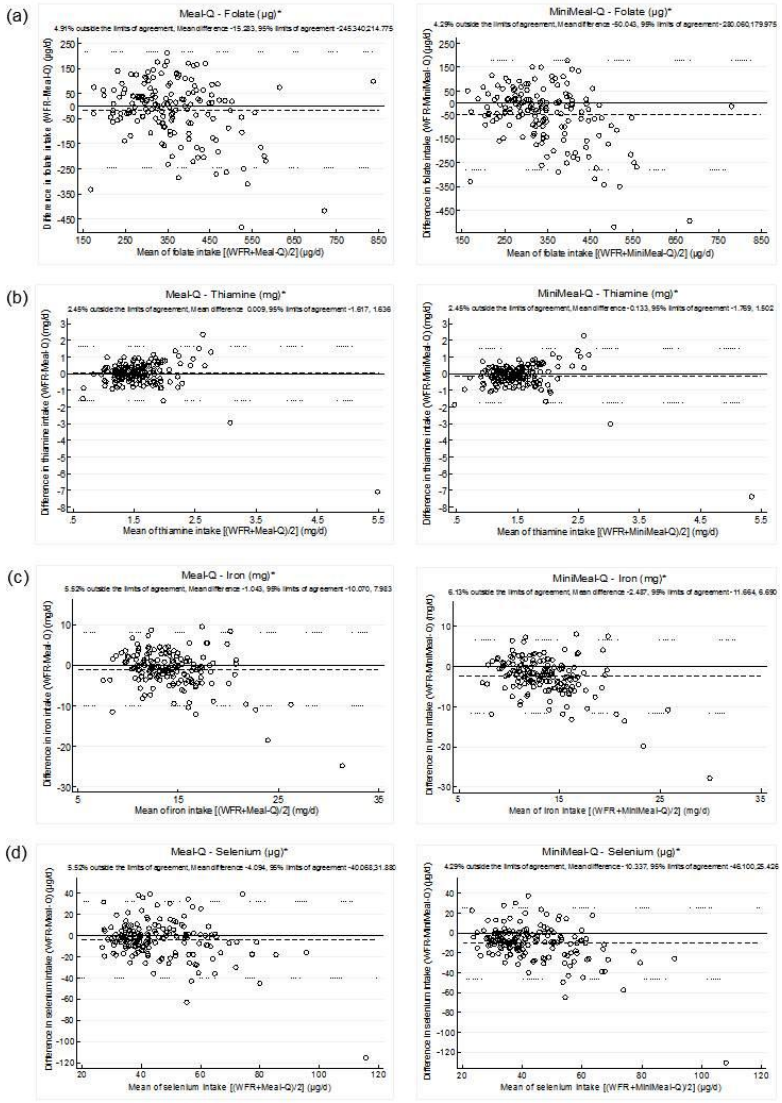

Figure 3. Bland-Altman plots with the WFR, Meal-Q and MiniMeal-Q for (a) folate, (b) thiamine, (c) iron and (d) selenium (n=163). Differences in intake between the WFR and the questionnaires are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). * Energy-adjusted.

Table 5 shows the Spearman correlation coefficients between Meal-Q, MiniMeal-Q and the WFR. Correlations for Meal-Q for crude intakes were in the range *r*=0.16-0.66. Excluding the statistically non-significant correlation for sodium, the energy-adjusted correlations for Meal-Q ranged from *r*=0.28 to *r*=0.67 and the de-attenuated correlations ranged from *r*=0.31 to *r*=0.69. The correlations were very similar for MiniMeal-Q, except for thiamine, which showed a stronger correlation with MiniMeal-Q than with Meal-Q.

Table 5 Spearman rank correlation coefficients between Meal-Q, MiniMeal-Q and the WFR (n=163).

	Spearman rank correlation coefficients[a] between questionnaires and the WFR							
	Meal-Q	MiniMeal-Q	Meal-Q	MiniMeal-Q	Meal-Q		MiniMeal-Q	
	Crude		Energy-adjusted		De-attenuated	(95% CI)	De-attenuated	(95% CI)
Beta-carotene[b]	0.51	0.51	0.46	0.46	0.51	(0.36-0.64)	0.51	(0.36-0.64)
Thiamine	0.33	0.37	0.28	0.35	0.35	(0.16-0.52)	0.43	(0.24-0.59)
Riboflavin	0.16	0.15	0.39	0.38	0.42	(0.27-0.55)	0.41	(0.26-0.54)
Niacin	0.39	0.37	0.43	0.42	0.47	(0.32-0.59)	0.46	(0.31-0.59)
Vitamin B6	0.39	0.40	0.32	0.32	0.35	(0.19-0.49)	0.35	(0.19-0.49)
Folate	0.50	0.50	0.50	0.50	0.53	(0.39-0.64)	0.53	(0.39-0.64)
Vitamin B12	0.39	0.28	0.46	0.37	0.51	(0.36-0.63)	0.41	(0.26-0.55)
Vitamin C	0.53	0.52	0.53	0·50	0.57	(0.36-0.64)	0.54	(0.41-0.65)
Vitamin D	0.34	0.32	0.31	0.30	0.36	(0.19-0.50)	0.34	(0.18-0.49)
Vitamin E	0.30	0.30	0.42	0.42	0.48	(0.32-0.61)	0.48	(0.33-0.61)
Calcium	0.23	0.22	0.29	0.24	0.31	(0.16-0.45)	0.25	(0.09-0.40)
Iron	0.44	0.43	0.42	0.38	0.46	(0.31-0.59)	0.42	(0.27-0.55)
Magnesium	0.52	0.52	0.54	0.52	0.56	(0.44-0.66)	0.54	(0.41-0.64)
Phosphorus	0.36	0.37	0.36	0.36	0.39	(0.24-0.52)	0.39	(0.24-0.52)
Potassium	0.42	0.42	0.41	0.38	0.43	(0.29-0.56)	0.40	(0.25-0.52)
Selenium	0.32	0.30	0.42	0.41	0.45	(0.30-0.57)	0.44	(0.30-0.57)
Sodium[c]	0.32	0.32	0.15	0.12	0.16	(-0.01-0.32)	0.14	(-0.04-0.30)
Zinc	0.33	0.34	0.31	0.31	0.34	(0.18-0.49)	0.35	(0.19-0.49)
Fiber	0.66	0.65	0.67	0.65	0.69	(0.60-0.77)	0.67	(0.57-0.75)

[a]All correlation coefficients were significant (*P* = .00-.04), except for energy-adjusted sodium (*P* = .06) and de-attenuated sodium assessed with Meal-Q as well as crude riboflavin (*P* = .06), energy-adjusted sodium (*P* = .12) and de-attenuated sodium assessed with MiniMeal-Q.

[b]n = 162 due to exclusion of one subject with implausibly high intake.

[c]n = 160 due to exclusion of three subjects with implausibly high intakes.

Reproducibility

Table 6 shows the absolute intake of micronutrients and fiber assessed with the two administered Meal-Q in group 2. There were no statistically significant differences between the questionnaires. The proportion of subjects classified into the same or adjacent quartile was 86-97% and in the extreme quartile 0-3% (Table 7). Crude ICCs were in the range *r*=0.45-0.85 and energy-adjusted ICCs in the range *r*=0.50-0.76 (Table 7).

Table 6 The median (IQR) daily micronutrient and fiber intake assessed with Meal-Q 1 and Meal-Q 2 from group 2, and the median (IQR) difference in intake between the questionnaires (n = 87)[a].

	Meal-Q 1		Meal-Q 2		Difference[b] Meal-Q 1 – Meal-Q 2	
	Median	(IQR)	Median	(IQR)	Median	(IQR)
Beta-carotene (µg)	3246	(2776)	2441	(3626)	126	(1271)
Thiamine (mg)	1.4	(0.7)	1.5	(0.9)	0.01	(0.48)
Riboflavin (mg)	1.7	(0.7)	1.6	(0.7)	0.03	(0.69)
Niacin (mg)	15	(9)	16	(9)	-0.06	(4.96)
Vitamin B6 (mg)	2.0	(1.0)	1.9	(1.0)	0.03	(0.67)
Folate (µg)	320	(187)	332	(218)	14	(105)
Vitamin B12 (µg)	3.7	(2.1)	4.0	(2.5)	-0.18	(1.44)
Vitamin C (mg)	108	(81)	99	(93)	-0.78	(41.10)
Vitamin D (µg)	4.9	(2.8)	5.2	(3.6)	-0.19	(3.32)
Vitamin E (mg)	9.6	(5.2)	9.5	(5.6)	0.27	(3.47)
Calcium (mg)	860	(449)	888	(454)	8.95	(299.27)
Iron (mg)	13	(8)	13	(10)	-0.07	(4.37)
Magnesium (mg)	406	(212)	418	(233)	10	(112)
Phosphorus (mg)	1419	(668)	1483	(518)	1.92	(430.63)
Potassium (mg)	3208	(1719)	3116	(1584)	38	(963)
Selenium (µg)	43	(23)	46	(23)	-0.12	(15.93)
Sodium (mg)	2466	(984)	2499	(1294)	-75	(757)
Zinc (mg)	11.0	(4.9)	11.0	(3.7)	0.004	(3.556)
Fiber (g)	28	(19)	25	(22)	0.20	(8.53)

[a]Missing values on Meal-Q 2 for four subjects.

[b]*P* = .07-.96 (Wilcoxon signed-rank test).

Table 7 Quartile cross-classifications of Meal-Q 1 and Meal-Q 2 from group 2, and crude and energy-adjusted intra-class correlation coefficients (ICC) (n=87)[a].

	Same quartile	Adjacent quartile	Same/adjacent quartile	Extreme quartile	Crude ICC		Energy-adjusted ICC	
	Percentage of subjects (%)				r_i	(95% CI)	r_i	(95% CI)
Beta-carotene (µg)	53	43	96	1	0.85	(0.79-0.91)	0.75	(0.66-0.84)
Thiamine (mg)	57	34	91	2	0.54	(0.40-0.69)	0.64	(0.51-0.76)
Riboflavin (mg)	59	33	92	3	0.45	(0.28-0.62)	0.63	(0.51-0.76)
Niacin (mg)	52	41	93	0	0.66	(0.54-0.78)	0.76	(0.67-0.85)
Vitamin B6 (mg)	53	36	89	1	0.49	(0.33-0.65)	0.50	(0.34-0.66)
Folate (µg)	59	38	97	1	0.71	(0.60-0.81)	0.73	(0.63-0.83)
Vitamin B12 (µg)	59	31	90	1	0.60	(0.47-0.74)	0.65	(0.53-0.78)
Vitamin C (mg)	52	43	95	1	0.80	(0.73-0.88)	0.74	(0.64-0.83)
Vitamin D (µg)	43	43	86	3	0.56	(0.42-0.70)	0.55	(0.41-0.70)
Vitamin E (mg)	57	34	91	0	0.73	(0.64-0.83)	0.73	(0.63-0.83)
Calcium (mg)	51	38	89	1	0.49	(0.33-0.65)	0.66	(0.54-0.78)
Iron (mg)	51	41	92	2	0.61	(0.47-0.74)	0.61	(0.48-0.74)
Magnesium (mg)	66	31	97	1	0.64	(0.51-0.76)	0.73	(0.64-0.83)
Phosphorus (mg)	54	33	87	3	0.46	(0.29-0.62)	0.62	(0.49-0.75)
Potassium (mg)	56	38	94	0	0.65	(0.52-0.77)	0.80	(0.73-0.88)
Selenium (µg)	61	33	94	1	0.64	(0.52-0.77)	0.72	(0.61-0.82)
Sodium (mg)	57	38	95	1	0.53	(0.38-0.68)	0.59	(0.45-0.72)
Zinc (mg)	46	40	86	1	0.50	(0.35-0.66)	0.63	(0.50-0.76)
Fiber (g)	55	39	94	0	0.77	(0.69-0.86)	0.71	(0.61-0.82)

[a]Missing values on Meal-Q 2 for four subjects.

DISCUSSION

Principal results

This validation study suggests Meal-Q and MiniMeal-Q to be useful tools for ranking micronutrient and fiber intake in epidemiological studies, with the exception of sodium. Furthermore, Meal-Q's reproducibility results indicate good assessment reliability.

Regarding assessment of absolute intake, both questionnaires underestimated intake of most micronutrients as compared to the WFR. This underestimation may partly be explained by the methodological differences between the methods. A food record has an open-ended design and is aimed to assess the whole diet during a consecutive number of days. In contrast, a questionnaire has predefined items and frequencies and naturally cannot assess the entire diet. Rather, the aim of a questionnaire is to assess dietary intake in a way that enables ranking of low to high consumers. Since risk comparisons in epidemiological studies commonly are made between different strata of intake, the ranking ability of dietary intake is usually of more interest than assessment of absolute intake [14,20]. We therefore conclude that Meal-Q and MiniMeal-Q are useful instruments in an epidemiological setting.

The captured between-person variance in intake assessed with MiniMeal-Q as compared to Meal-Q demonstrated only a minor loss of information when using MiniMeal-Q despite its approximately 30% fewer food items. This indicates MiniMeal-Q to be a valuable

alternative when a shorter questionnaire is desirable.

Acknowledging that the evaluation of MiniMeal-Q is made with truncated Meal-Q data, comparison between them should be interpreted carefully. Comparing our results to other validation studies should also be done with caution given that differences in study design and subject demographics may affect the results. Yet, bearing its limits in mind, such comparisons, which are commonly made, are crucial in evaluating a questionnaire's performance.

Comparison with prior work

The cross-classifications with the WFR showed both questionnaires to yield ranking agreements comparable to or better than other similar validation studies [21-25], of which two evaluated web-based FFQs. The highest ranking agreement for Meal-Q and MiniMeal-Q was seen for fiber with 89-90% placed into the same or adjacent quartile, which is higher than in some other studies [21,23-24]. The lowest ranking agreement was seen for sodium, as has been shown previously [21,23], and which likely reflects the difficulty in assessing salt intake.

The Bland-Altman plots showed that Meal-Q and MiniMeal-Q generally had difficulties in precision as seen in the large variance. This varying bias over the intake range was also indicated by the limits of agreement, which for some nutrients deviated from 5%. For most nutrients the questionnaires performed less well in assessing high intakes. This might be explained by a limitation of food items, excessive grouping of several food items on each row, lack of high frequency alternatives or the use of standard portion sizes for many food items. The overall large variance seen for most nutrients could arise from various sources, e.g. a limited frequency range of the questionnaires and/or a high between-person variation in the WFR. Although the Bland-Altman method has been recommended for use in validation studies, it should be noted that we would not expect an absolute agreement between the questionnaires and the WFR due to their inherent methodological differences. Instead, the plots are helpful in assessing the magnitude of the inaccuracy and detecting potential varying bias. Despite the varying bias over the intake range seen in the Bland-Altman plots, the cross-classifications of quartiles indicated that both Meal-Q and MiniMeal-Q were able to yield a good ranking ability.

The limited number of studies using Bland-Altman plots for assessment of micronutrient validity and that some of them used log-transformed values makes comparisons with our results difficult. However, two other studies have also detected varying bias over the intake range; Labonté *et al* showed similar results in variance for fiber intake [23], while Pinto *et al* showed a larger variance for folate and iron intake than seen in the present study [25].

The energy-adjusted and de-attenuated Spearman correlation coefficients in the current study were similar to or better than correlations obtained in other validation studies with comparable study design [21-27]. Sodium showed a statistically non-significant correlation with the WFR for both questionnaires, which has also been seen previously [21,23]. Furthermore, in a review of 392 validation studies of vitamin intake, Henríquez-Sánchez *et al*. showed mean correlations between a FFQ and a dietary record in the range r=0.41-0.53 [28]. Another review

of 109 validation studies of iron, calcium, selenium and zinc reported mean correlations between a FFQ and a dietary record ranging from *r*=0.36 to *r*=0.60 [29]. Both reviews show that the correlations in our study are in line with other validation studies for most nutrients, with the exception for thiamine, riboflavin, vitamin B6, vitamin D, vitamin E, calcium and zinc which correlations were somewhat lower in the present study. Merely correlations for vitamin B12, niacin, riboflavin, vitamin E, calcium, magnesium, selenium and fiber improved after energy adjustment, a phenomenon also seen before [25,26]. A possible explanation for this is varying correlation with energy between different nutrients [14], which is a feature that also depends on the population.

The use of correlation coefficients in validation studies is extensive but has been criticized since they only measure a relationship and not the agreement between two methods [15]. However, as mentioned, we would not expect an absolute agreement between a FFQ and a food record since FFQs are designed to rank individuals rather than to assess absolute intake [20]. In this way, the correlation coefficient is a useful measure of validity since it assesses the ranking ability.

The sodium intake assessed with Meal-Q and MiniMeal-Q only included salt in food items and dishes in the nutrient database. Both questionnaires have a yes/no-question regarding salt in cooking and table salt, however since it is difficult to estimate amounts, this information was not included in the nutrient calculations. The WFR could potentially capture added salt, however this was only reported for a minor fraction of all food items. Hence the sodium assessed with the questionnaires and the WFR both originate from salt already present in food items and dishes from the nutrient database. The low validity for sodium could therefore best be explained by a general large random variation in assessment between the questionnaire and the WFR.

The reproducibility of Meal-Q indicated that it performed well in its reliability to rank dietary intake, with a high proportion of subjects in the same/adjacent quartile and a low proportion of misclassified subjects. The quartile cross-classifications were comparable to Labonté *et al* [23]. Energy-adjusted correlations between repeated FFQs have generally ranged between *r*=0.5-0.8 in other studies [30] and Meal-Q showed quite similar results. The ICCs for fiber, vitamin E, vitamin B6, niacin, vitamin C, beta-carotene, folate, magnesium and potassium in the present study were on average lower than those found by Schröder et al. [31]. Furthermore, the ICCs were slightly lower than the Pearson correlations found by Labonté *et al* [23], yet higher than the Pearson correlations found by Pinto *et al* [25].

Limitations and strengths

A strength of this study include the large sample size for this type of validation study. Moreover, there was a low drop-out and high compliance for the assessment methods throughout the entire study. The high compliance probably reflects a well-motivated study population, something that is vital for the study's internal validity. The motivation might arise from a general higher interest in health among self-selected subjects as compared to invited subjects. Furthermore, subjects with nutritional background might also be more motivated than those without this background. It should be acknowledged that the young and mainly female study population might have

implications on external validity. Regarding data handling, the web-based format of the questionnaires and the WFR minimized potential errors in the conversion of crude consumption data into nutrient intakes. Web-based formats have previously shown to improve the data quality [4,32].

In the validation of a dietary assessment method the reference method should have measurement errors independent from those of the test method. Since the WFR is an open-ended prospective method and a FFQ is a retrospective method with pre-defined food items and frequencies, dependent measurement errors are less likely to occur. Nevertheless, both methods are, as all dietary assessment methods, susceptible to social desirability. This could affect them in similar ways and thereby increasing their error dependency. Also, both methods are linked to the same nutrient database. Therefore a validation study of a dietary assessment method should be evaluated in terms of relative validity rather than absolute validity. The present study did unfortunately not have the means to include an objective reference method for micronutrient intake as biomarkers (e.g. urinary potassium, thiamine and sodium), which would have been a valuable complement to the WFR.

Meal-Q and MiniMeal-Q reflects dietary intake during the past few months, while the WFR captures dietary intake over seven consecutive days; hence a perfect agreement should not be expected. Ideally, the WFR would have been performed repeatedly over a longer time period to better mirror the assessment aim of the questionnaires. Also, for the reproducibility analysis, the second Meal-Q should preferably have been administered after a slightly longer time period to decrease the influence from the first questionnaire. However, time constraints made a longer validation study impossible. In the comparison between questionnaires and the WFR, adjustments for within-person variance in the WFR were made to minimize the effect of day-to-day variations in intake. Furthermore, MiniMeal-Q should ideally have been evaluated in a separate validation study, however this was not possible due to time constraints.

Conclusions

This validation study demonstrated that Meal-Q and MiniMeal-Q are useful questionnaires for ranking micronutrient and fiber intake in epidemiological studies using web-based data collection. However, assessment of sodium intake requires further attention in future questionnaire versions. Furthermore, the reproducibility results showed Meal-Q to have a good reliability. It should be noted that the study was conducted in a young, mainly female and well educated study population and that MiniMeal-Q would merit its own validation study.

Acknowledgements

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AP, LL, KB: interpretation of results; SEC: drafted the manuscript; SEC, EM, SEB, AP, OB, LL, KB: review and revision of the manuscript including approval of final version.

Conflicts of interest

None of the authors had a personal or financial conflict of interest

Abbreviations

BMI: body mass index

DLW: doubly labeled water

FFQ: food frequency questionnaire

ICC: intra-class correlation coefficient

PAL: physical activity level

WFR: weighed food record

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MULTIMEDIA APPENDIX LEGENDS

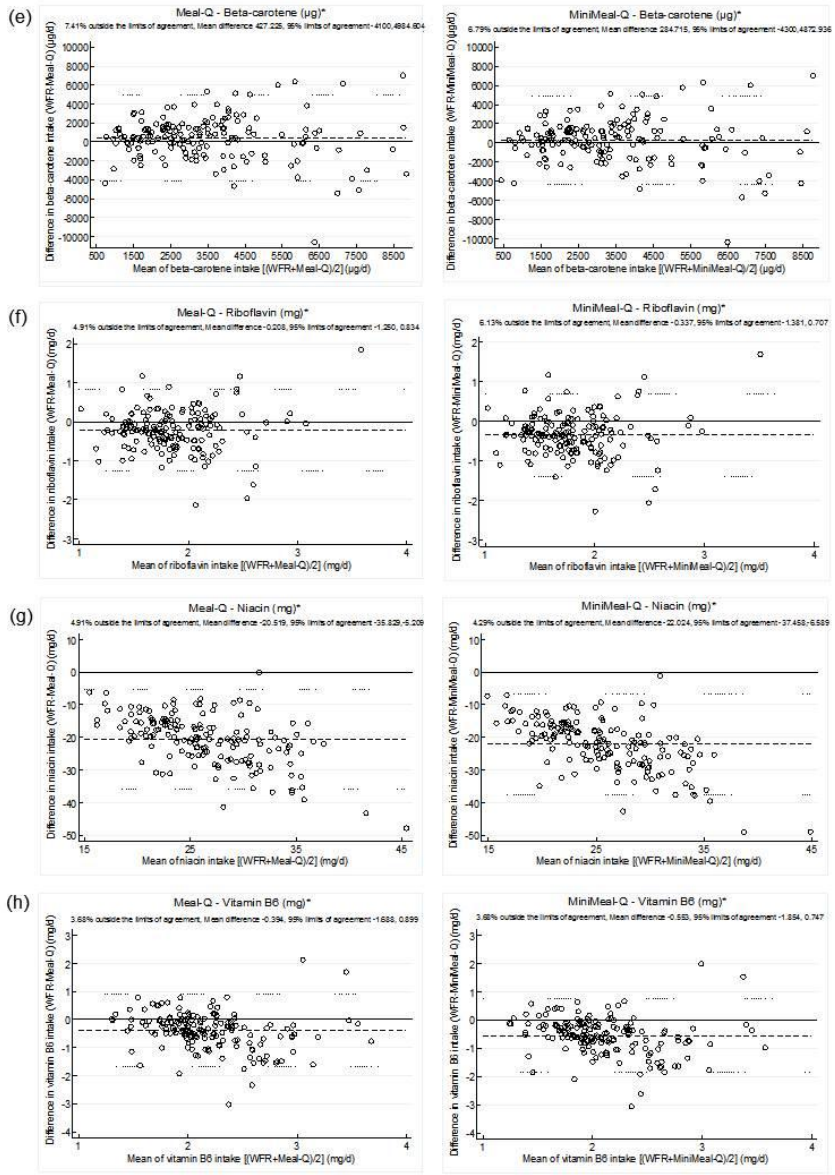
Multimedia Appendix 1 Bland-Altman plots with the WFR, Meal-Q and MiniMeal-Q for (e) beta-carotene (n=162 (due to exclusion of one subject with implausibly high intake)), (f) riboflavin, (g) niacin and (h) vitamin b6 (n=163). Differences in intake between the WFR and the questionnaires are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). * Energy-adjusted.

Multimedia Appendix 2 Bland-Altman plots with the WFR, Meal-Q and MiniMeal-Q for (i) vitamin B12, (j) vitamin C, (k) vitamin D and (l) vitamin E (n=163). Differences in intake between the WFR and the questionnaires are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). * Energy-adjusted.

Multimedia Appendix 3 Bland-Altman plots with the WFR, Meal-Q and MiniMeal-Q for (m) calcium, (n) magnesium, (o) phosphorus and (p) potassium (n=163). Differences in intake between the WFR and the questionnaires are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). * Energy-adjusted.

Multimedia Appendix 4 Bland-Altman plots with the WFR, Meal-Q and MiniMeal-Q for (q) sodium (n=160 due to exclusion of three subjects with implausibly high intakes), (r) zinc and (s) fiber (n=163). Differences in intake between the WFR and the questionnaires are plotted against the mean of the two methods. The long-dashed line shows the mean difference. The short-dashed lines show the 95% limits of agreement (mean difference ±2 SD). * Energy-adjusted.

MULTIMEDIA APPENDIX 1



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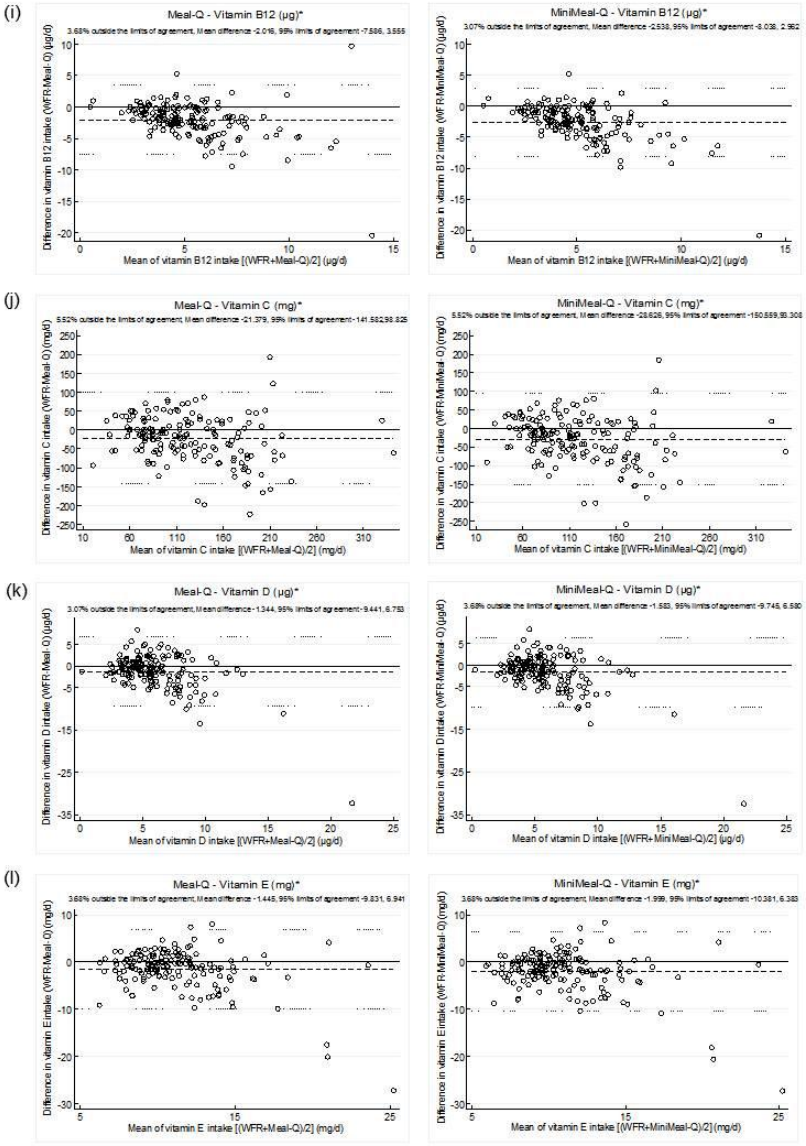

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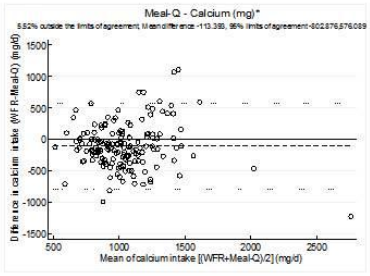

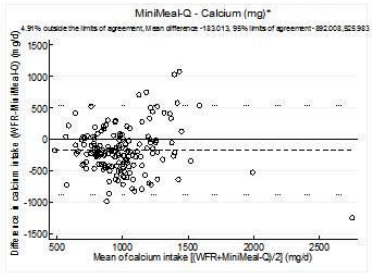

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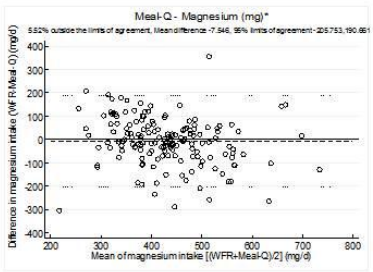

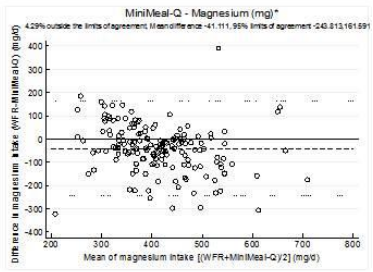

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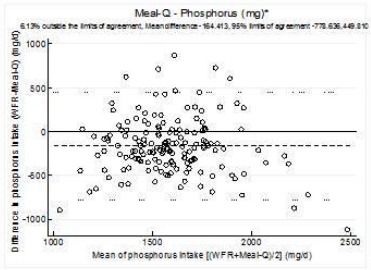

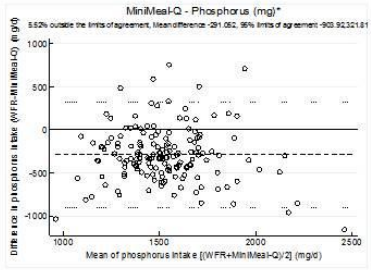

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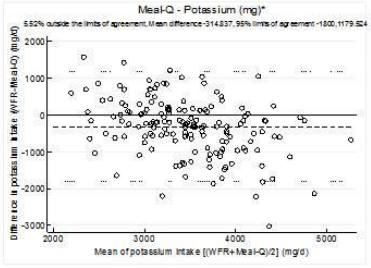
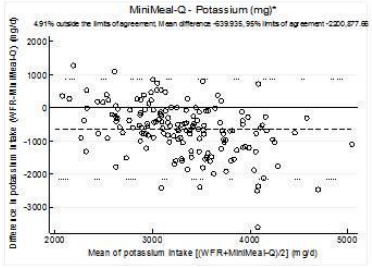
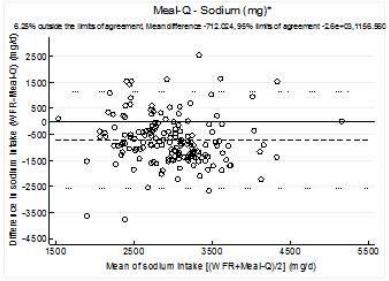
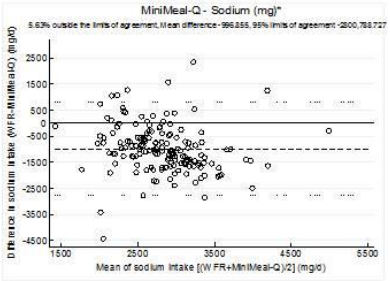




MULTIMEDIA APPENDIX 4

(q)





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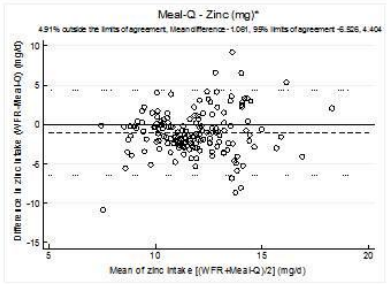

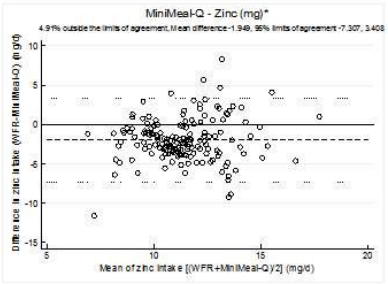

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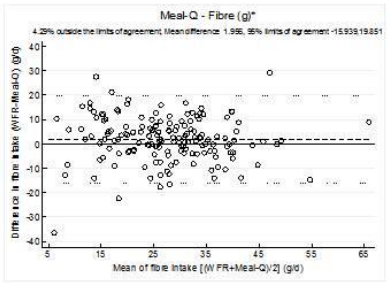

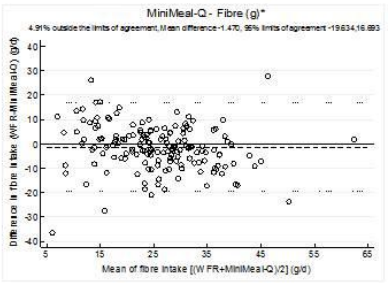

III

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Adherence to the Nordic Nutrition Recommendations as a measure of a healthy diet and upper respiratory tract infection

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Abstract

Objective: The Nordic countries have published joint dietary recommendations, the Nordic Nutrition Recommendations (NNR), since 1980. We evaluated adherence to the NNR as a measure of a healthy diet and its potential association with self-reported upper respiratory tract infection (URTI).
Design: A prospective, population-based study with a follow-up period of 4 months. Dietary intake was assessed using a semi-quantitative FFQ with ninety-six items, along with other lifestyle factors, at baseline. URTI was assessed every three weeks. A Poisson regression model was used to control for age, sex and other confounding factors.
Setting: A middle-sized county in northern Sweden.
Subjects: Swedish men and women (*n* 1509) aged 20–60 years.
Results: The NNR include recommendations on macronutrient proportions, physical activity and intake of micronutrients, sodium, fibre and alcohol. We found that overall adherence to the NNR was moderately good. In addition, we found that high adherence to the NNR (>5·5 adherence points) was not associated with a lower risk of URTI (incidence rate ratio (IRR) 0·89, 95% CI 0·73, 1·08) compared with low adherence (<4·5 adherence points). When investigating individual components of the NNR, only high physical activity was associated with lower URTI risk (IRR = 0·82, 95% CI 0·69, 0·97) whereas none of the dietary components were associated with risk of URTI.
Conclusions: Overall adherence to the NNR was moderately good. Overall adherence to the NNR was not associated with URTI risk in our study. However, when investigating individual components of the NNR, we found that high physical activity was associated with lower URTI risk.

Keywords
Upper respiratory tract infection
Epidemiology
Nutrition recommendations

The first nutrition recommendations date back to Britain in 1862. Following economic depression, the British Privy Council wanted guidelines for food that was cheap but would avoid diseases related to malnutrition[1]. Today, most industrialized countries have nutrition recommendations and the Nordic countries have published joint recommendations, the Nordic Nutrition Recommendations (NNR)[2], since 1980. The NNR focus on the energy distribution from macronutrients, intake of micronutrients, and the most recent version from 2004 includes physical activity. The NNR are based on the current nutritional situation in the Nordic countries and available scientific knowledge to promote overall good health and reduce the risk of diet-associated diseases.

Upper respiratory tract infection (URTI), including the common cold and influenza, is a frequent disease which, in addition to causing individual suffering, is responsible for huge costs to society. URTI is estimated to cost $US 40 billion per year in the USA[3], not including the cost of influenza, and is the most common reason for seeking primary care in many countries[4]. Despite this, little is known about how to decrease susceptibility.

A large number of studies have focused on the effects of dietary patterns on chronic diseases, such as CVD and cancer[5–9]. For example, a lower risk of CVD was found among women adhering to the Dietary Guidelines for Americans[9]. Less is known about the effects of dietary patterns on respiratory infections. Most nutrient deficiencies are related to suppression of both innate and adaptive immune functions. For example, the fat-soluble vitamins A, D and E have been shown to affect immune cell lineage development, as well as several immune functions including apoptosis and phagocytosis[10]. In addition, vitamin E supplementation in the elderly has been shown to reduce susceptibility to infection[11], and vitamin C has since 1937 been suggested to reduce the

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risk of URTI[12]. Other nutrients suggested to affect the risk of URTI are *n*-3 fatty acids, selenium and zinc[10,13,14]. However, less is known about macronutrient composition and risk of URTI.

We aim to evaluate adherence to the NNR in a population-based, prospective web cohort and to investigate if high adherence to the NNR is associated with self-reported incidence of URTI, compared with low adherence.

Because URTI typically has a brief duration, frequent follow-ups are needed for an accurate incidence tally. We assessed self-reported incidence of URTI every third week for 15 weeks by sending an email linked to a short web questionnaire to all participants.

Subjects and methods

The LIME (Lifestyle and Immune function) study comprises 1509 men and women, aged 20–60 years, residing in a middle-sized county in northern Sweden. The study is a population-based cohort on lifestyle factors and immune function, using the Internet as a tool for data collection. Subjects were randomly selected from the Swedish Total Population Registry at Statistics Sweden, and invited to participate in the study in January 2004. The study was approved by the Research Ethics Committee at Karolinska Institutet.

Study design

Invitations to participate in the study were sent out via regular paper mail. The invitations included information on how to access the web questionnaire, details on use of a web browser, the URL to the web questionnaire, and an individual username. The baseline questionnaire about lifestyle factors included a question about the participant's email address. We sent five follow-up questionnaires during the following 15 weeks (in February, March, early April, late April and May). Every questionnaire included questions on URTI during the three preceding weeks. We sent reminders to non-responders by email 1·5 weeks after each follow-up.

Five thousand individuals were invited to participate in this prospective study, of whom 1805 completed the baseline web questionnaire in the three weeks that the web questionnaire was open. No further reminder for the baseline questionnaire was given since we aimed for a high continuation rate rather than a high initial response rate. After exclusions, 1509 were eligible for follow-up questionnaires. We excluded participants who had URTI at baseline (*n* 236), lacked an email address (*n* 17) or chose not to disclose it (*n* 20). During the study it became evident to us that participants at one specific workplace had an email server that filtered our emails as junk mail. We excluded these participants from the study (*n* 23) since they could not be invited via email to fill out the follow-up questionnaires. Response rate for each follow-up questionnaire ranged between 83 and 84 % (number of responders to that follow-up questionnaire divided by the number eligible for that follow-up questionnaire). In total, 1111 out of 1509 responded (74 % of baseline respondents) to all five follow-ups.

The baseline web questionnaire has been described in detail elsewhere[15]. In brief, the questionnaire included immediate checks for incomplete or implausible answers, reminder messages to the respondent when a question was left unanswered, hiding of non-relevant follow-up questions, automatic summarization of answers, voluntary personalized feedback to the respondents on BMI, energy expenditure and intakes of vitamin C, calcium, fibre and iron, and illustrations to clarify complex questions. No software installation was required to complete the web questionnaire.

Assessment of exposure

Diet was assessed at baseline by a ninety-six-item validated semi-quantitative FFQ[16,17], including questions on vitamin and mineral supplements, measuring the usual dietary intake. Consumption data from the questionnaire, including portion size, were linked to the Swedish National Food Administration database version 19/05/2009 (Uppsala, Sweden) on energy and nutrient content in various food products in order to calculate the daily mean intake. Comparing the FFQ with a 7 d food diary[16], Spearman correlation coefficients ranged from 0·38 (iron) to 0·81 (vitamin C) for micronutrients and had the following values for macronutrients: 0·44 (protein), 0·73 (carbohydrates), 0·71 (fibre), 0·70 (total fat), 0·75 (SFA), 0·66 (MUFA), 0·49 (PUFA) and 0·81 (alcohol). Total physical activity was also assessed at baseline. Participants were asked to estimate the amount of time (hours and minutes) spent on each out of nine activity levels on a usual day and night. Each activity level was explained by examples of activities[18].

Description of the Nordic Nutrition Recommendations

For several decades, the Nordic countries have worked together to set dietary guidelines. The NNR are based on research data from epidemiological studies and laboratory studies, and the recommendations apply to all the Nordic countries: Sweden, Norway, Denmark, Finland and Iceland. The NNR include recommendations on total energy intake, macronutrients as a percentage of total energy intake, intakes of fibre and salt, as well as recommended daily intakes of vitamins and minerals and recommendations on physical activity. The main goal for the NNR is to set guidelines to promote good health and to prevent major chronic diseases on a population basis[2].

Categorization of adherence

Table 1 lists the NNR by six groups of individual recommendations: intake from (i) macronutrients (e.g. total fat, protein and carbohydrates), (ii) micronutrients (vitamins

Table 1 The individual recommendations in the Nordic Nutrition Recommendations (NNR) and their criteria for cut-offs and adherence scores; and mean intakes and adherence in the study population: Swedish men and women (*n* 1509) aged 20–60 years, LIME (Lifestyle and Immune function) study, 2004

	Cut-offs and adherence scores		Mean intakes and adherence		
Recommendation in NNR	Recommended intake	Total score	Mean intake	SD	% of participants within NNR intake
Macronutrient recommendation (% of energy)					
Saturated fat	≤10	0–1	11·4	2·5	28
Monounsaturated fat	10–15		9·4	1·8	36
Polyunsaturated fat	5–10		3·8	0·8	7
Essential fatty acids	≥3		3·7	0·8	83
Total fat	25–35		26·8	4·4	64
Protein	10–20		18·0	2·6	81
Carbohydrates	50–60		55·2	5·6	64
Sugar	≤10		6·8	2·7	88
Micronutrient recommendation					
Vitamin recommendations for men*					
Retinol equivalents (mg/d)	≥0·9	0–1	0·5	0·4	17
Thiamin (mg/d)	≥1·4		1·4	0·5	60
Riboflavin (mg/d)	≥1·7		1·7	0·7	60
Niacin (mg/d)	≥19		16·0	6·2	38
Vitamin B_6 (mg/d)	≥1·6		2·1	0·7	86
Folate (mg/d)	≥0·3		0·3	0·1	27
Vitamin B_{12} (μg/d)	≥2		5·6	3·4	95
Vitamin C (mg/d)	≥75		114·0	82·7	61
Vitamin D (μg/d)	≥7·5		4·2	2·2	8
α-Tocopherol (mg/d)	≥10		6·4	2·3	13
Mineral recommendations for men*					
Ca (g/d)	≥0.8		1·1	0·4	70
P (g/d)	≥0.6		1·5	0·5	99
Mg (g/d)	≥0·35		0·4	0·1	62
K (g/d)	≥3·5		3·3	1·1	45
Fe (mg/d)	≥9		12·1	4·4	45
Zn (mg/d)	≥9		10·9	3·8	81
Se (μg/d)	≥50		33·3	13·8	16
Iodine (not measured)	–		–	–	–
Cu (not measured)	–		–	–	–
Other recommendations					
Fibre (g/d)	>25	0–1	25·1	10·9	42
Sodium recommendation for men (g/d)*	≤2·8	0–1	2·7	0·9	53
Alcohol recommendation (% of energy)	≤5	0–1	3·4	3·1	78
Physical activity recommendation†					
Moderate activity (min/d) and/or	≥60	0–1			79
Vigorous activity (min/d)	≥30				
Total score (min–max)		0–6			

*Different cut-offs were used for women for some vitamins and minerals[2].
†Moderate activity was defined as activities corresponding to 5 MET and vigorous as activities >6 MET (where MET is metabolic equivalent task). The NNR advises more than 30 min and preferably more than 60 min of moderate and/or vigorous physical activity daily in addition to inactive living.

and minerals), (iii) sodium, (iv) alcohol, (v) fibre and (vi) physical activity. For each major NNR group, every individual recommendation variable was graded on a continuous scale from 0 to 1 as follows. 1 point was given to intakes within the NNR; 0 point was given to intakes below a defined lowest value and/or above a highest value (for nutrient variables, the median of the ten lowest and/or ten highest intakes among the study population was used; for physical activity, <30 min/d was used as lower value); and a relative score of 0–1 points was awarded for intakes or activity levels between the recommendation level and above the defined highest and lowest values according to the following calculation (where Y is the new adherence variable and X is the intake or activity level).

For lower limits, Y varies from 0 to 1:

$$Y = (X - \text{lowest value})/(\text{lower NNR cut-off point} - \text{lowest value})$$

For upper limits, Y varies from 1 to 0:

$$Y = 1 - [(X - \text{higher NNR cut-off point})/(\text{highest value} - \text{higher NNR cut-off point})]$$

Summation of the scores of individual recommendations into groups of recommendation (e.g. fat, carbohydrates, vitamins and minerals) was made by summing up the individual scores and dividing the sum by the number of individual recommendations included in the group; in that way equal weight was given to each group in the final adherence score. Sodium, alcohol, fibre and physical

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activity were kept as individual recommendations. Scores from the six recommendation groups were then summarized into a total score ranging from 0 to 6 points for each person, and divided into three groups of adherence using pre-chosen arbitrary cut-off points: <4·5 points for low adherence (range 0·17–4·49, median 4·10), 4·5–5·5 points (median 4·99) for medium adherence and >5·5 points (range 5·50–5·83, median 5·60) for high adherence. The group with low adherence was considered as the reference group in the subsequent analyses.

Moreover, three additional scoring models were tested in order to evaluate the effect of scoring per se. The first alternative scoring model differs from the initial model with regard to giving more weight to the score for vitamins and minerals. The second scoring model differs from the initial model by giving more weight to the scores for vitamins, minerals and individual macronutrients. Finally, the third alternative scoring model differs from the initial model by excluding recommendations for total fat intake, monounsaturated fat intake, polyunsaturated fat intake, protein intake and carbohydrate intake and only by including recommendations that are open-ended (no closed intervals; e.g. ≤10 % of energy from saturated fat). This way, measurement error of the FFQ would have less influence on the results.

Ascertainment of upper respiratory tract infection

Self-reported URTI was assessed at baseline and in all five follow-up questionnaires. In the follow-up questionnaires, participants were asked if they currently had an infection (cold or influenza) or if they had had a new infection during the last three weeks, or since the last questionnaire. Participants were considered to have URTI if they answered 'yes' to this question. They were instructed not to count a URTI episode twice, even if it crossed over two follow-up periods. Follow-up questions about symptoms were given to all participants who reported an infection. Seven symptoms were recorded: sore throat, cough, runny nose, headache, malaise, fever and unspecified symptoms. The influenza season of 2003–2004 in Sweden was of medium intensity. Activity peaked during the last week of December 2003 and the first week of January 2004, but declined shortly after and remained low during the rest of the season, which ended in the middle of March[19]. Influenza vaccination history was collected at baseline and updated at every follow-up. Allergy symptoms were collected in the follow-up questionnaires in April and May (the pollen season in the part of the country where the study population lived), since symptoms of pollen allergy can mimic URTI.

Data analysis methods

We divided the number of reported URTI by person-time at risk to get incidence rates. We estimated incidence rate ratios (IRR) with 95 % confidence intervals using Poisson regression models to assess and control for age and sex along with other confounding factors. Disease-free participants contributed three weeks of time under risk for each 3-week follow-up period. A participant with no reported URTI could therefore contribute up to five 3-week periods of time at risk, a total of 15 person-weeks. Since we did not know exactly when an episode of URTI occurred or how long it lasted during the 3-week period, we assigned to participants who reported URTI 1·5 weeks of risk time out of the 3-week follow-up period. We considered each follow-up period to be independent of previous or later periods for the same person. The correlation in the data due to repeated measurements on the same individual was investigated by using GEE (generalizing estimating equations), but no evidence of substantial correlation was found and GEE were not used in the final analysis. Therefore, we assumed that the risk of contracting URTI was independent of one's previous history of URTI.

We assessed the confounding potential of a large number of potential confounding factors by eliminating one factor at a time from the full model. We included in the final Poisson model those that changed the effect estimate more than 10 % when dropped. Age, sex, BMI, asthma, self-reported weakened immune system, month, energy intake and education all had small confounding effects, but nevertheless were kept in all multivariable models. Perceived stress and smoking status changed the estimates by more than 10 % in all NNR adherence score models and were included in multivariable analyses. We also assessed as potential confounders snuff use, contact with small children, contact with large crowds at leisure time, use of public transport, poor sleep, influenza vaccination history and pollen allergy. None of these changed the estimate by more than 10 % and was not included in the final model. Individuals with missing information on the NNR score or any covariate in the final multivariable model were excluded from the analyses (n 254). The multivariable analyses on the initial NNR adherence scoring model included 15 780 person-weeks contributed by 1255 participants, generating 1090 cases combined (0–5 cases per participant).

We fit a cubic spline model for the initial NNR adherence scoring model with six knots (at minimum and maximum, and at adherence scores of 1·0, 2·0, 3·0 and 4·0)[20] to characterize any shape of a possible dose–response relationship. We present a graph of the rates at the baseline value for all other covariates predicted from the model.

The analyses were conducted using SAS version 9·2 (SAS Institute, Cary, NC, USA) and Stata Intercooled version 10·1 (Stata Corporation, College Station, TX, USA) statistical software packages.

Results

We found only small differences in baseline characteristics between study participants regarding age, sex, BMI, asthma and energy intake. Higher adherence to the NNR

Table 2 Baseline characteristics of study participants by adherence to the Nordic Nutrition Recommendations (NNR): Swedish men and women (*n* 1509) aged 20–60 years, LIME (Lifestyle and Immune function) study, 2004

	Adherence to the NNR (initial scoring model) (score 0–6)					
	Low adherence <4·5 points		Medium adherence 4·5–5·5 points		High adherence >5·5 points	
No. of participants	488		687		175	
	%		%		%	
Age (years)						
20–29	33		31		29	
30–39	26		27		21	
40–49	19		19		24	
50–60	22		23		27	
Sex (male)	41		47		47	
BMI						
Low–normal (<25 kg/m^2)	56		56		60	
High–very high (>25 kg/m^2)	44		44		40	
Chronic stress level						
Low (<23, below median)	47		55		58	
High (>23, above median)	53		45		42	
Education						
Secondary school or less	37		37		46	
University	63		64		55	
Smoking						
Current smoker	18		14		11	
Previous smoker	31		28		22	
Never smoker	32		58		67	
Asthma (yes)	7		8		11	
	Mean	SD	Mean	SD	Mean	SD
Energy intake (kJ/d)	7261	2582	7801	2101	7834	1559
Carbohydrate intake (g/d)	224	84	244	66	251	50
Protein intake (g/d)	75	33	81	26	80	17
Saturated fat intake (g/d)	21	9	23	8	23	7

appeared to be associated with lower perceived stress, lower education level and fewer smokers (Table 2).

Adherence to each dietary recommendation is shown in Table 1. PUFA and vitamin D showed the least adherence: 7% of participants were within the recommended interval for PUFA and only 8% reached the recommended intake level for vitamin D. Mean intake for PUFA was 3·8 (SD 0·8) % of total energy intake, which is well below the recommended intake of 5–10% of energy. Observed intake for vitamin D was also well below the recommended intake of 7·5 μg/d, with an observed mean of 4·2 (SD 2·2) μg/d. Participants also scored lower for α-tocopherol (13%) and selenium (16%), where the mean intake was 6·4 (SD 2·3) mg/d and 33·3 (SD 13·8) μg/d, respectively, much lower than the recommended intakes of 10 mg/d (for men) and 50 μg/d (for men), respectively (Table 1). A previous validation study for dietary variables of the FFQ found that Spearman correlations were moderate for PUFA (0·49) and vitamin D (0·48), low for α-tocopherol (0·37) and high for selenium (0·72)[16]. Almost all participants (99%) reached the recommended intake level of phosphorus of 0·6 g/d and almost as many (95%) reached the recommended intake of vitamin B_{12} of 2 μg/d. For phosphorus and vitamin B_{12}, the mean intakes (1·5 (SD 0·5) g/d and 5·6 (SD 3·4) μg/d) were almost three times higher than the recommended intakes (Table 1).

We found that 50% of study participants had medium adherence (4·5–5·5 points) and 13% had high adherence (>5·5 points) to the NNR according to the initial scoring model (Table 2). Using the initial scoring model, high adherence to the NNR (>5·5 points) was not associated with lower risk of URTI compared with low adherence (<4·5 points; IRR = 0·89, 95% CI 0·73, 1·08; Table 3). In order to evaluate the effect of different scoring models for adherence, we tested three additional scoring models, see Table 3. However, the results did not change much. This may partially be explained by the correlation coefficients between the initial scoring models and the alternative scoring models being relatively high, as expected, ranging from 0·75 to 0·85.

Using the same scoring criteria as the initial model we excluded those with daily or weekly multivitamin use (about 12% of all participants; IRR = 0·93, 95% CI 0·76, 1·15), but the results were similar to the initial model including all multivitamin supplement users for high adherence to the NNR (>5·5 points) compared with low adherence (<4·5 points).

Adherence to the different groups of recommendations, according to the initial scoring model, and risk of URTI is shown in Table 4. We found no association between URTI and adherence to any of the groups of recommendations. The carbohydrate group (including

Table 3 Initial and alternative scoring models of adherence to the Nordic Nutrition Recommendations (NNR) and risk of upper respiratory tract infection among Swedish men and women (*n* 1509) aged 20–60 years, LIME (Lifestyle and Immune function) study, 2004

	Adjusted for age and sex				Multivariable model	
	Cases	Person-weeks	IRR	95 % CI	IRR*	95 % CI
Adherence to NNR, initial scoring model† (score 0–6)						
Low (<4·5 points)	242	3285	1·00	–	1·00	–
Medium (4·5–5·5 points)	686	9867	0·91	0·80, 1·03	0·88	0·77, 1·01
High (>5·5 points)	206	3246	0·93	0·78, 1·12	0·89	0·73, 1·08
Adherence to NNR, alternative scoring model 1‡ (score 0–7)						
Low (<5·0 points)	155	2293·5	1·00	–	1·00	–
Medium (5·0–6·0 points)	518	7038	1·02	0·88, 1·18	0·98	0·83, 1·15
High (>6·0 points)	461	7066·5	0·97	0·83, 1·14	0·91	0·76, 1·09
Adherence to NNR, alternative scoring model 2§ (score 0–9)						
Low (<7·0 points)	319	4456·5	1·00	–	1·00	–
Medium (7·0–8·0 points)	590	8451	0·92	0·81, 1·04	0·88	0·77, 1·01
High (>8·0 points)	225	3490·5	0·96	0·81, 1·13	0·89	0·74, 1·08
Adherence to NNR, alternative scoring model 3‖ (score 0–8) (only including macronutrient recommendations with open-ended cut-offs)						
Low (<6·0 points)	144	2043	1·00	–	1·00	–
Medium (6·0–7·0 points)	472	6393	1·03	0·89, 1·20	0·98	0·83, 1·16
High (>7·0 points)	518	7962	0·98	0·84, 1·15	0·91	0·76, 1·09

IRR, incidence rate ratio.
*IRR for all adherence scores were adjusted for age (20–29, 30–39, 40–49 and 50–60 years), sex, energy intake (in four categories), BMI (low, normal, overweight and obese), weakened immune system (yes/no), asthma (yes/no), perceived stress (below and above median), education level (secondary school or less and university), smoking (daily/less frequent/previous/never) and month (February to May).
†Scoring was calculated by awarding 1 point (1p) for adherence to the following recommendation subgroups in NNR: physical activity (1p), fibre intake (1p), sodium intake (1p), alcohol intake (1p), mineral and vitamin intake (1p), and macronutrient intake (1p).
‡Scoring was calculated by awarding 1 point for adherence to each of the following recommendation subgroups in NNR: physical activity (1p), fibre intake (1p), sodium intake (1p), alcohol intake (1p), mineral (1p) and vitamin intake (1p), and macronutrient intake (1p).
§Scoring was calculated by awarding 1 point for adherence to the following recommendation subgroups in NNR: physical activity (1p), fibre intake (1p), sodium intake (1p), alcohol intake (1p), mineral (1p) and vitamin intake (1p); and 1 point to each subgroup in macronutrient recommendations: carbohydrate and sugar intake (1p), total fat intake, saturated fat intake, essential fat intake, polyunsaturated fat intake, monounsaturated fat intake (1p) and protein intake (1p).
‖Scoring was calculated by awarding 1 point for adherence to open-ended (no intervals) recommendation subgroups in NNR: physical activity (1p), fibre intake (1p), sodium intake (1p), alcohol intake (1p), mineral (1p) and vitamin intake (1p); and 1 point to each subgroup in macronutrient recommendations; sugar intake (1p), saturated fat intake, essential fat intake (1p) and protein intake (1p).

sugar), protein group, physical activity group and alcohol group were not included in Table 4, since >75 % of participants fulfilled the recommendations for these groups and would therefore make comparisons difficult.

In Table 5 we show associations between URTI and absolute intake of individual recommendations, rather than adherence score, for macronutrients, sodium, fibre and physical activity. We found no association for any individual group, except for physical activity, which was associated with reduced risk of URTI (IRR = 0·82, 95 % CI 0·69, 0·97). Single vitamins and minerals were not included in the analysis due to the large number of individual vitamins and minerals.

To study the possible continuous relationship between adherence to the NNR and URTI risk, we fit spline regressions for the initial scoring model (shown in Fig. 1). We also fit spline regressions for each group of individual NNR and URTI risk. Only physical activity appeared to be associated with URTI risk (Fig. 2).

Discussion

We found that high adherence to the NNR was not associated with risk of URTI, compared with low adherence. Different scoring models were used to assess adherence to the NNR, but the result did not differ between the scoring models. However, when analysing different parts of the NNR score, we found that high physical activity was associated with lower risk of URTI but none of the recommendations on diet were associated with risk of URTI. The study also demonstrates that the overall adherence to the NNR was moderately good.

To the best of our knowledge, there are no other studies available that have studied the effect of dietary recommendations on the risk of URTI. Previous studies evaluating dietary recommendations have focused on the effect on diseases such as myocardial infarction or cancer. McCullough *et al.*[5,6] evaluated the adherence to the Dietary Guidelines for Americans (DGA) using ten equally weighted groups (e.g. servings of grain, fruit and meat). They found no preventive effect for cancer and no effect on CVD in women[6] and only a small reduced risk for men[5]. A more recent study, using a continuous scoring model similar to ours, found an association between DGA and atherosclerosis progression in women[9]. It is possible that the use of food groups in recommendations such as DGA is more efficient in predicting disease than the NNR which emphasize macronutrient proportions.

In the present study, diet was assessed by a validated semi-quantitative FFQ. However, we acknowledge the limitations of FFQ. The FFQ does not assess the entire

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Table 4 Adherence score (0–1) for each group of recommendations of the Nordic Nutrition Recommendations (NNR) and risk of upper respiratory tract infection among Swedish men and women (*n* 1509) aged 20–60 years, LIME (Lifestyle and Immune function) study, 2004

Categorization of adherence score for each group of recommendation of the NNR*	Adjusted for age and sex				Multivariable model	
	Cases	Person-weeks	IRR	95 % CI	IRR†	95 % CI
Fat group‡ intake (adherence score, 0–1)						
Low (<0·50)	267	3457·5	1·00	–	1·00	–
Medium (0·50–0·60)	487	6898·5	0·97	0·84, 1·13	0·96	0·82, 1·12
High (>0·60)	404	6354	0·92	0·78, 1·08	0·91	0·78, 1·08
Vitamin group intake (adherence score, 0–1)						
Low (<0·60)	414	5772	1·00	–	1·00	–
Medium (0·60–0·90)	539	7918·5	0·98	0·86, 1·12	0·93	0·78, 1·10
High (>0·90)	205	3019·5	1·05	0·89, 1·25	0·97	0·77, 1·24
Mineral group intake (adherence score, 0–1)						
Low (<0·75)	277	4045·5	1·00	–	1·00	–
Medium (0·75–0·95)	558	7938	1·07	0·93, 1·24	1·08	0·90, 1·30
High (>0·95)	323	4726·5	1·11	0·94, 1·30	1·08	0·85, 1·37
Sodium intake (adherence score, 0–1)						
Low (<0·80)	288	3870	1·00	–	1.00	–
Medium (0·80–<1·00)	252	3645	0·96	0·81, 1·13	0·97	0·80, 1·18
High (1·00)	618	9195	0·92	0·80, 1·06	0·93	0·74, 1·17
Fibre intake (adherence score, 0–1)						
Low (<0·50)	245	3394·5	1·00	–	1·00	–
Medium (0·50–1·00)	450	6432	1·00	0·86, 1·17	0·97	0·82, 1·14
High (1·00)	463	6883·5	1·03	0·88, 1·20	0·96	0·80, 1·16

IRR, incidence rate ratio.
*Carbohydrate group (including sugar), protein, physical activity and alcohol were not included in this table since >75 % of participants were within recommendations (adherence score = 1) for those groups.
†IRR for all adherence scores were adjusted for age (20–29, 30–39, 40–49 and 50–60 years), sex, energy intake (in four categories), BMI (low, normal, overweight and obese), weakened immune system (yes/no), asthma (yes/no), perceived stress (below and above median), education level (secondary school or less and university), smoking (daily/less frequent/previous/never) and month (February to May).
‡Including total fat intake, saturated fat intake, essential fat intake, polyunsaturated fat intake and monounsaturated fat intake.

Table 5 Absolute cut-off points for individual recommendations of the Nordic Nutrition Recommendations (NNR) and risk of upper respiratory tract infection among Swedish men and women (*n* 1509) aged 20–60 years, LIME (Lifestyle and Immune function) study, 2004

Intake of individual recommendations of the NNR*	Adjusted for age and sex				Multivariable model	
	Cases	Person-weeks	IRR	95 % CI	IRR†	95 % CI
Saturated fat intake (% of energy)						
<10	315	4636·5	1·00	–	1·00	–
10–12	393	5485·5	1·07	0·92, 1·24	1·09	0·93, 1·27
>12	450	6588	1·03	0·89, 1·19	1·05	0·90, 1·22
Protein intake (% of energy)						
<17	421	5815·5	1·00	–	1·00	–
17–20	533	7615·5	1·02	0·89, 1·16	1·02	0·89, 1·16
>20	204	3279	0·94	0·79, 1·12	0·94	0·78, 1·11
Carbohydrate intake (% of energy)						
<50	195	2806·5	1·00	–	1·00	–
50–60	725	10 780·5	0·93	0·79, 1·09	0·92	0·78, 1·09
>60	238	3123	0·98	0·80, 1·19	0·96	0·78, 1·17
Sodium intake (mg/d)						
<2000	313	4258·5	1·00	–	1·00	–
2000–3800	661	9925·5	0·96	0·83, 1·10	0·87	0·71, 1·06
>3800	184	2526	1·01	0·83, 1·23	0·85	0·63, 1·14
Alcohol intake (% of energy)						
<2·5	586	8094	1·00	–	1·00	–
2·5–5·0	328	4896	0·99	0·86, 1·14	0·99	0·86, 1·14
>5·0	244	3720	1·02	0·88, 1·19	1·05	0·87, 1·12
Fibre intake (g/d)						
<25	695	9826·5	1·00	–	1·00	–
25–35	302	4518	1·02	1·17, 1·04	0·99	0·86, 1·15
>35	161	2365·5	1·04	0·88, 1·24	0·98	0·80, 1·19
Moderate‡ and/or vigorous physical activity (min/d)						
<60	522	7062	1·00	–	1·00	–
60–120	281	3940·5	0·90	0·78, 1·05	0·89	0·77, 1·05
>120	378	5982	0·86	0·74, 1·01	0·82	0·69, 0·97

IRR, incidence rate ratio.
*Single vitamins or minerals were not studied since they were too many in number and would increase the risk of chance findings.
†IRR for all adherence scores were adjusted for age (20–29, 30–39, 40–49 and 50–60 years), sex, energy intake (in four categories), BMI (low, normal, overweight and obese), weakened immune system (yes/no), asthma (yes/no), perceived stress (below and above median), education level (secondary school or less and university), smoking (daily/less frequent/previous/never) and month (February to May).
‡Moderate physical activity cut-offs: <2 h, 2–3 h, >3 h.

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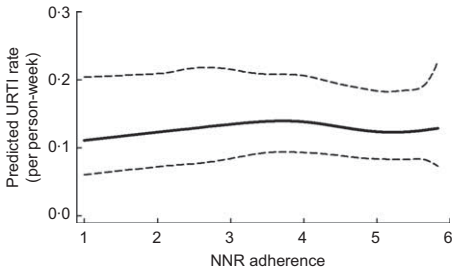

Fig. 1 Spline regressions for overall adherence to the Nordic Nutrition Recommendations (NNR) expressed as adherence score and risk of upper respiratory tract infection (URTI) among Swedish men and women (*n* 1509) aged 20–60 years, LIME (Lifestyle and Immune function) study, 2004. ---, 95 % confidence intervals

diet, but large parts of the dietary intake. The shortcomings of an FFQ can blunt a true association[21] and thus the results from the current study should be interpreted with caution. For example, in the European Prospective Investigation into Cancer and Nutrition, which used both FFQ and food diaries, a stronger association was found between saturated fat and breast cancer on data from food diaries compared with FFQ[22]. In addition, a previous validation study using the same FFQ and repeated records of total dietary intake found that the correlation coefficients between FFQ and dietary records were moderate to high for different nutrients (ranging from 0·31 for iron to 0·81 for vitamin C). Still, in dietary surveys, regardless of dietary assessment method, fat (especially saturated fat) and carbohydrates are considered to be typically under-reported, while protein is typically over-reported[23]. Obese and weight-conscious

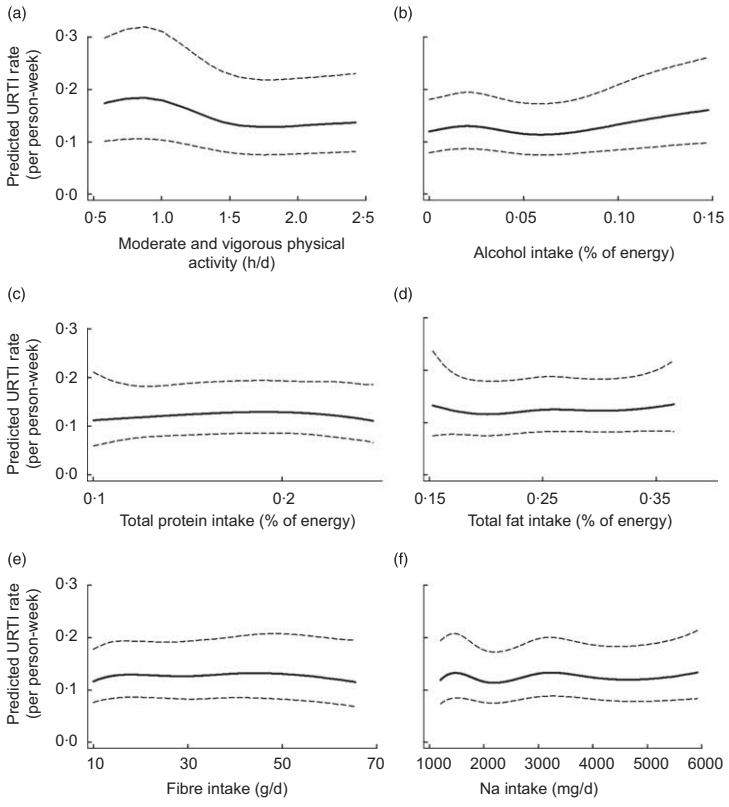

Fig. 2 Spline regressions for groups of individual recommendations in the Nordic Nutrition Recommendations (NNR) using absolute intake and risk of upper respiratory tract infection (URTI) among Swedish men and women (*n* 1509) aged 20–60 years, LIME (Lifestyle and Immune function) study, 2004. ---, 95 % confidence intervals

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participants are considered to be those who under-report unhealthy food the most[24–26]. The intakes of various nutrients in the present study were of the same magnitude as intakes reported in a previous national Swedish study, Riksmaten, from 1997–1998, of 1215 Swedish men and women[27]. In Riksmaten, the participants were asked to record their total intake of food and beverages using a 7 d food dairy and did not use an FFQ. A more precise measure of some dietary intakes would have been to use biomarkers, which were not available in our study.

Moderate validity for certain food items or nutrients may result in bias, with double-ended macronutrient recommendations (e.g. 50–60 % of energy from carbohydrates) being more vulnerable to poor validity (i.e. misclassification when using FFQ). In alternative scoring model 3 we excluded all double-ended recommendations, but the results did not change much compared with the initial scoring model. In order to reduce bias from misclassification using absolute cut-off points for the NNR scoring, we used a continuous score proportional to the distance from the guidelines (in all scoring models). By doing this, intakes close to, but not within the recommended intake were still given a high score.

We cannot rule out the possibility that the null results for overall adherence to the NNR in the present study are due to participants being generally very well-nourished, leading to a limited variation and skewing towards higher adherence scores for certain recommendations in order to see an association with URTI. Nevertheless, small variations in the scoring were taken into account by using a continuous grading scale for intakes close to NNR cut-offs. Our results on physical activity and URTI are supported by previous studies which have suggested a protective association on URTI of moderate levels of physical activity[28], possibly mediated by a higher circulating number of natural killer cells found in resting athletes[29]. Although we cannot rule out a potential effect of reverse causality, to minimize this possibility we assessed exposure before outcome (i.e. baseline) and thereafter excluded those with URTI at baseline.

Major strengths of our study include its population-based design and the comprehensiveness of case identification using frequent follow-ups. Also, the continuation rate for each follow-up was high, i.e. more than 80 % of the participants responded to each follow-up web questionnaire. Incidence of URTI was self-reported and self-diagnosis of URTI has been shown to be reliable in adult patients[30]. The prospective nature of the study makes any potential recall bias highly unlikely. We adjusted for several potential confounders in the model, including stress, smoking, education level and season, observing no great difference between the age-adjusted and the multivariable models, but unmeasured aspects of a healthy diet or lifestyle (e.g. hand-washing) might result in residual confounding.

In conclusion, we found that the overall adherence to the NNR was moderately good. Overall adherence to the NNR was not associated with URTI risk in our study. However, when investigating individual components of the NNR, we found that high physical activity was associated with lower URTI risk. In addition, we found that the feasibility of using a web questionnaire at baseline and sending follow-up questionnaires via email was high in this population.

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IV

Intake of vitamin C, vitamin E, selenium, zinc and polyunsaturated fatty acids and upper respiratory tract infection: a prospective cohort study

Original article

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Keywords: antioxidants, polyunsaturated fatty acids, upper respiratory tract infection, cohort study, epidemiology

Running head: antioxidants, PUFA and upper respiratory tract infection

ABSTRACT

Background

Both antioxidants and polyunsaturated fatty acids (PUFAs) play a role in our immune defense and may also affect the susceptibility to upper respiratory tract infection (URTI). We here present a prospective cohort study to examine dietary vitamin C, vitamin E, selenium, zinc and PUFAs and supplement intake in relation to URTI.

Subjects and methods

A total of 1,533 Swedish women and men aged 25-64 years were followed for nine months during 2011-2012. Information on dietary and supplement intake was assessed through a web-based food frequency questionnaire and events of URTI were self-reported prospectively as they occurred. Cox proportional hazards regression was applied to obtain incidence rate ratios with 95% confidence intervals.

Results

The incidence rate ratios (95% CI) of URTI for dietary intake among women were 0.69 (0.55-0.88) for vitamin C, 0.77 (0.62-0.96) for vitamin E and 0.57 (0.39-0.83) for docosahexaenoic acid (DHA), comparing high to low intake. A possible beneficial effect could also be seen for arachidonic acid (AA) (0.80 (0.65-0.99)). No protective association for any nutrient from diet could be seen among men, instead an increased risk of URTI with medium vitamin E (1.42 (1.09-1.85)) and high zinc intake (1.50 (1.04-2.16)). We did not find any association between supplement intake and URTI.

Conclusions

This study showed that high intake of vitamin C, vitamin E, DHA and possibly also AA was associated with decreased URTI incidence among women. Medium vitamin E and high zinc intake was associated with an increased risk of URTI among men.

INTRODUCTION

The most common illness among humans is acute upper respiratory tract infection (URTI), or the common cold, and adults on average have 2.5 infections per year[1]. During 1995-2006, nearly 52 million annual ambulatory visits occurred in the United States due to acute respiratory infections[2], and the American annual cost for work loss is estimated to be above $20 billion[3]. Despite the individual and societal economic burden of URTI, little is known about how to decrease susceptibility to the disease.

The nutrient status is known to affect the immune system and inadequate nutrient intake will weaken most innate and adaptive immune responses[4,5]. Lately, research on antioxidants and PUFAs has received more focus. A recent meta-analysis on trials using vitamin C supplements showed an overall reduced risk for the common cold among athletes training in the subarctic regions and a borderline significant protective effect in the general population[6]. The effect of vitamin E on URTI has been studied in trials on elderly and in male smokers, however the results have been diverging[7-9]. Selenium deficiency is associated with repression of resistance to infections and selenium status has been shown to affect the immune response to influenza virus in mice and human cells[10,11]. Moreover, a meta-analysis of randomized controlled trials on zinc supplementation found lower incidence rate ratios among adults[12]. Although a previous cohort study did not show any association between URTI and dietary vitamin C or zinc intake[13], a more recent cohort study showed protective associations between URTI and dietary vitamin C intake among women as well as a potential protective association with supplement intake of vitamin C and E among men[14].

Both AA and n-3 fatty acids are abundant in immune cells and the content can be altered through dietary intake[15]. AA can be transformed into eicosanoids, which act in inflammatory processes[16,17], whereas n-3 fatty acids can form anti-inflammatory resolvins[15]. Although PUFAs have been shown to affect the immune function, to our knowledge, no studies have so far investigated the association between PUFA intake and URTI.

Although previous studies have been conducted on antioxidants and URTI, most of them have been trials focusing on supplement intake and specific populations such as elderly, children or athletes. Here we present a population-based cohort study of 1,533 subjects followed over nine months and with data on dietary intake of vitamin C and E, selenium, zinc and PUFAs as well as supplement intake and their association with URTI incidence.

SUBJECTS AND METHODS

In summer 2011, a total of 14,008 individuals residing in Eskilstuna municipality in Sweden were invited to participate in the prospective cohort study SWEDE-I; Studies of Work Environment and Disease Epidemiology-Infections. The sampling frame was created by Statistics Sweden by cross linkage between its Labor Market Register and the Total Population Register to create an age- and gender

stratified random sample. Eligible individuals were working full-time or part-time while people working from home, unemployed, retired, students and those on long sick or parental leave were ineligible. A total of 2,450 individuals agreed to participate, whereof 188 did not meet the study criteria for participation and 25 were excluded due to administrative errors. In total, 2,237 women and men aged 25-64 years were enrolled in the study whereof 1,533 with complete data on dietary intake. The Research Ethics Committee at Karolinska Institutet approved the study.

Study design

Invitations to participate were sent out by regular mail in summer 2011. Thereafter, the enrollment started on August 22nd with just over nine months of follow-up until May 31st 2012. A subject contributed person-time from their entry into the study up until the end of or loss to follow-up. The mean (SD) follow-up time was 32 (15) weeks. A study scheme of SWEDE-I can be seen in **Figure 1**. Registration to participate was self-administered and made through either a secure web site, interactive voice response telephone service (IVRS) or through a postal response form.

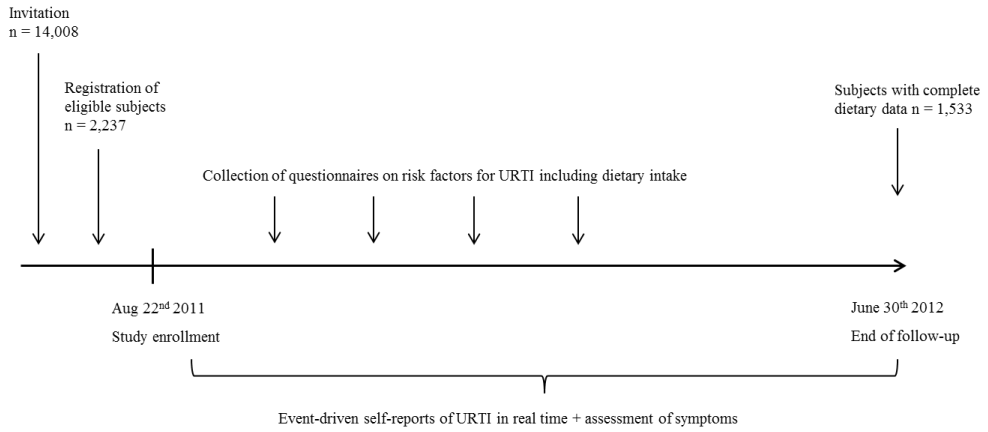

Figure 1 The study scheme of SWEDE-I.

SWEDE-I included five questionnaires for exposure data collection. The first four were available on either web or paper while the fifth questionnaire was available only on the web. The subjects voluntarily chose to fill out questionnaire 1-4 either on the web or on paper. They covered demography information and questions about the work place, family, social contacts and health status including potential determinants for exposure to and transmission of viral infections. The fifth questionnaire assessed dietary intake and physical activity. The paper questionnaires were sent out one by one at approximately one-month intervals while all web-questionnaires were available

to fill out at study start directly after registration. The participants were followed passively and they reported URTI episodes on an event-driven basis via a secure website or an automated IVRS telephone service.

Exposure assessment

Dietary and supplement intake was assessed through a validated web- and meal-based food frequency questionnaire, MiniMeal-Q[18]. Energy-adjusted and de-attenuated Spearman correlation coefficients between MiniMeal-Q and a 7-day weighed food record were *r*=0.54 for vitamin C, *r*=0.48 for vitamin E, *r*=0.44 for selenium and *r*=0.35 for zinc[19]. The energy-adjusted and de-attenuated Pearson correlation coefficient for PUFAs was *r*=0.40[18]. MiniMeal-Q includes 75-126 food items and is interactive with follow-up and skip patterns, which adapts the questionnaire to the respondents' dietary habits. It asks about habitual dietary intake and assesses intake of food items, dishes, beverages, energy, nutrients and the use of dietary supplements. A total of 1,533 subjects completed MiniMeal-Q with a majority (80%) having filled out the questionnaire within five months from study start. Dietary data was linked to the nutrient database of the Swedish National Food Agency[20]. Calculation of daily mean intake of energy and nutrients was made through a nutrient calculation program developed and validated by the research group (MiniMealCalc).

Nutrient intake from food

Energy-adjusted nutrient intake from food was categorized per sex and into three arbitrary levels (low, medium and high intake) according to the intake distribution with the lowest level used as reference category. For women, the categories for vitamin C were: <45, 45-110, >110 (mg/d); for vitamin E: <6.5, 6.5-9.5, >9.5 (mg/d); for selenium: <25, 25-45, >45 (µg/d); for zinc: <7.5, 7.5-11, >11 (mg/d); for omega-6: <0.45, 0.45-1.25, >1.25 (g/d); for AA: <40, 40-85, >85 (mg/d); for omega-3: <0.13, 0.13-0.5, >0.5 (g/d); for eicosapentaenoic acid (EPA): <30, 30-100, >100 (mg/d); and for docosahexaenoic acid (DHA): <0.1, 0.1-0.32, >0.32 (g/d). For men, the categories for vitamin C were: <40, 40-100, >100 (mg/d); for vitamin E: <6, 6-8.5, >8.5 (mg/d); for selenium: <25, 25-40, >40 (µg/d); for zinc: <7, 7-10, >10 (mg/d); for omega-6: <0.4, 0.4-1.2, >1.2 (g/d); for AA: <40, 40-80, >80 (mg/d); for omega-3: <0.13, 0.13-0.45, >0.45 (g/d); for EPA: <25, 25-100, >100 (mg/d); and for DHA: <0.1, 0.1-0.35, >0.35 (g/d).

Supplement intake

Dietary supplement use of vitamin C and/or multivitamin/minerals as well as of omega-3 fatty acids was categorized into two categories: daily/weekly or monthly/in period/no use with the latter used as reference category. Supplement use of vitamin E, selenium and zinc was rare and therefore not evaluated.

Potential confounding factors and effect measure modification

We evaluated a number of potential confounding factors for the association between dietary and supplement intake and URTI: body mass index (BMI; weight in kg/height in m^2), educational level (upper secondary school or less and university), smoking (daily smoking during last month

or no smoking), physical activity level (PAL), having small children (0-6 years) in the household, flu vaccination (proceeding year), chronic illness (heart disease, high blood pressure, diabetes, rheumatism, kidney disease or tumor treated by doctor), sleep quality and duration, weakened immune system (treated by doctor) and pulmonary disease (treated by doctor). For dietary intake, we also evaluated intake of alcohol, beta-carotene, folate and iron. The following supplements were also evaluated; vitamin C, D, E and A, antioxidant, multivitamin/mineral, selenium, zinc, omega-3, folate and iron. For supplement intake and its association with URTI, we evaluated the following nutrients from dietary intake: PUFAs, vitamin C and E, selenium, zinc, beta-carotene, folate and iron. Further, supplement intake of antioxidants, vitamin E, selenium, zinc, vitamin A, vitamin D, folate and iron was also considered. For each studied nutrient and supplement, we mutually evaluated the confounding potential of other studied nutrients and supplements. Effect measure modification regarding dietary and supplement intake was evaluated for all potential confounding factors.

Ascertainment of URTI

Each URTI event was self-reported throughout the study at the time it occurred. Subjects were instructed to report, on their own initiative, as soon as they believed that they had a cold. Reporting was done through either IVRS via regular telephone or a web-based form where the participants responded to a set of questions regarding date of onset and presence of symptoms including sore throat, coughing, runny nose, headache, fever and body ache. The criterion for defining an URTI event was the presence of runny nose, sore throat or coughing or the presence of either two or all three symptoms. An URTI event with onset date at least four days after a previous URTI was considered a new event. The first two weeks of follow-up for each subject were truncated, i.e. the follow-up on URTI started 14 days after study entry (to have time to send out nasal swabs for virus testing, yet these results are not covered in this study). Reminders in the form of newsletters were sent out on six occasions during the study.

Statistical analysis

Demographic and descriptive statistics were presented for all subjects as well as by sex. Statistically significant differences in nutrient intake between sexes were tested using Wilcoxon-Mann-Whitney test and Kruskal-Wallis test was used to compare nutrient intake between categories of age, BMI, education, smoking and physical activity. Difference in supplement intake between sexes was tested using the Fisher's exact test. The level of statistical significance was set to $\alpha=0.05$. Log or square root transformation of non-normally distributed nutrients was made prior to energy adjustment with the residual method[21,22]. Smoothed hazard estimates (URTI events/person-week) with 95% confidence intervals were obtained for women and men. Furthermore, the number of reported URTI was divided by person-time at risk to retrieve incidence rates. Incidence rate ratios (IRR) with 95% confidence intervals (CI) were obtained using the Cox proportional hazards regression model with the lowest intake

group as reference category. Results were obtained separately for women and men by including an interaction term between sex and the exposure. Possible dependency between URTI events was controlled for using the robust sandwich estimator for standard errors.

Potential confounding factors were evaluated by forward addition to each model. A covariate was considered a confounder if changing the estimate with ≥10%. Age, BMI, education and energy all had small confounding effects, but were nevertheless kept in the model since they were statistically significantly associated with URTI and suggested to be confounders according to the literature. Effect measure modification was evaluated by including an interaction term between the main exposure and the covariate and interaction with time was evaluated by including a time-varying covariate in the model. Since pollen allergy can mimic URTI symptoms, all models were also run without subjects reported having pollen allergy to evaluate if the estimates changed. The time point of filling out MiniMeal-Q was also evaluated for potentially affecting the reported intake and thereby the estimates. The tested time periods were Aug-Sept, Oct-Nov, Dec-Feb, March-May and June. Subjects with missing information on any covariate were excluded from the final multivariate models: n=74, whereof 62 women and 12 men.

Restricted cubic spline models[23,24] with three evenly distributed knots were used to graphically evaluate a potential dose-response relationship between dietary intake and URTI while controlling for confounding factors.

The statistical analyses were performed using STATA statistical software version 13.1 (StataCorp LP, College Station, TX, USA).

RESULTS

There were small differences in subject characteristics between women and men (**Table 1**). More men than women were overweight, while women were more likely to have a university degree than men. Women reported a slightly higher crude vitamin C intake from food than men, while men reported a somewhat higher crude intake of selenium and zinc.

Table 1. Characteristics of study subjects[a].

	All subjects, n=1533	Women, n=926	Men, n=607
Age (years); %			
25-34	12	13	11
35-44	32	32	32
45-54	27	29	25
55-64	29	27	32
Body Mass Index[b]; %			
Normal weight	45	52	35
Overweight	36	27	48
Obese	14	14	15
Education[c]; %			
Upper secondary school or less	40	32	52

University	59	67	48
Current smokers[d]; %	11	12	8.9
Asthma[e]; %	9.8	11	7.9
Immunodeficiency disease/reduced immune defence[f]; %	1.3	1.4	1.2
Chronic illness[g]; %	20	19	21
Physical activity level (PAL); median (IQR)	1.80 (1.54;2.07)	1.82 (1.57; 2.05)	1.77 (1.48; 2.14)
Energy and nutrient intake[h]; median (IQR)			
Energy intake; kJ	6820 (5271;9038)	6543 (5146; 8570)	7201 (5693; 9681)
Vitamin C intake; mg	69 (44;103)	73 (47; 105)	68 (40; 100)
Vitamin E intake; mg	7.8 (5.7;10.5)	7.9 (5.7; 10.6)	7.7 (5.7; 10.2)
Selenium intake; mcg	35 (26;46)	34 (26; 44)	37 (28; 48)
Zinc intake; mg	9.2 (7.2;12.2)	8.9 (7.0; 11.8)	9.7 (7.5; 12.8)
Omega-6 intake; g	0.70 (0.41;1.22)	0.70 (0.40; 1.20)	0.69 (0.42; 1.23)
Arachidonic acid intake; mg	58 (40;80)	57 (39; 79)	59 (42; 82)
Omega-3 intake; g	0.23 (0.12;0.46)	0.23 (0.12; 0.46)	0.22 (0.12; 0.46)
EPA intake; mg	49 (33;104)	45 (33; 104)	52 (34; 105)
DHA intake; g	0.18 (0.11;0.32)	0.17 (0.11; 0.32)	0.18 (0.11; 0.32)

[a]Wilcoxon-Mann-Whitney test was used to test statistical significant differences in nutrient intake between sexes. Kruskal-Wallis test was used to compare nutrient intake between categories of age, BMI, education, smoking and physical activity. Fisher's exact test was used to compare differences in supplement intake between sexes.
[b]Body Mass Index categories, normal weight: <25; overweight: 25-30; obese: >30. One man and 10 women were underweight (BMI <18.5). Missing information on BMI for 8 men and 58 women.
[c]Missing information on education for 5 men and 6 women.
[d]Defined as having been smoking daily during the last month. Missing information on smoking for 2 men and 3 women.
[e]Asthma treated by a doctor (yes/no). Missing information on asthma for 13 men and 19 women.
[f]Immunodeficiency disease or reduced immune system treated by a doctor (yes/no). Missing information for 16 men and 22 women.
[g]Chronic illness (heart disease, high blood pressure, diabetes, rheumatism, kidney disease or tumor) treated by a doctor (yes/no). Missing information on chronic illness for 22 men and 30 women.
[h]Not adjusted for energy intake.

Table 2 shows the mean (SD) daily energy-adjusted nutrient intake from food categorized into low, medium and high intake as well as frequency of supplement intake. A higher proportion of women than men consumed vitamin C supplements daily or weekly, while intake of multivitamin/minerals and omega-3 fatty acids was similar between the sexes.

Table 2. Mean (SD) daily nutrient intake[a] from food categorized into low, medium and high intakes and supplement use categorized into daily/weekly, monthly and sporadic use.

	Women, n=926						Men, n=607					
	Low Intake; Mean (SD)	n	Medium intake; Mean (SD)	n	High intake; Mean (SD)	n	Low Intake; Mean (SD)	n	Medium intake; Mean (SD)	n	High intake; Mean (SD)	n
Vitamin C, mg	33.1 (8.4)	193	74 (18)	552	158 (57)	172	27.6 (8.7)	160	66 (17)	325	140 (57)	113
Vitamin E, mg	5.6 (0.80)	233	7.8 (0.81)	481	11.5 (2.4)	203	5.2 (0.80)	145	7.1 (0.71)	340	10.2 (2.2)	113
Selenium, µg	19.4 (4.8)	188	34.2 (5.5)	551	59 (15)	187	19.0 (5.2)	164	31.9 (4.2)	295	50 (12)	146
Zinc, mg	6.4 (1.1)	200	9.1 (0.95)	551	13.0 (2.3)	175	5.8 (1.3)	125	8.4 (0.85)	332	11.5 (1.5)	148
Omega-6 fatty acids, g	0.31 (0.10)	200	0.78 (0.23)	521	2.1 (1.0)	205	0.30 (0.10)	122	0.70 (0.21)	343	2.3 (1.5)	142
Arachidonic acid intake; mg	30 (7.6)	224	60 (12)	512	116 (37.8)	189	28 (9.1)	140	59 (11)	338	116 (40.7)	129
Omega-3 fatty acids, g	0.082 (0.032)	174	0.27 (0.10)	571	0.85 (0.46)	181	0.082 (0.034)	136	0.24 (0.082)	339	0.95 (0.67)	132
EPA; mg	0.015 (0.010)	213	0.054 (0.019)	490	0.14 (0.06)	223	0.012 (0.010)	136	0.053 (0.017)	327	0.16 (0.10)	144
DHA; g	0.06 (0.03)	175	0.19 (0.06)	539	0.46 (0.18)	212	0.06 (0.03)	133	0.19 (0.06)	353	0.50 (0.20)	121
Supplement use; n (%)	Daily/weekly		Monthly		Sporadic		Daily/weekly		Monthly		Sporadic	
Vitamin C	49 (5.3)		18 (1.9)		80 (8.6)		20 (3.3)		19 (3.1)		29 (4.8)	
Multivitamin/mineral	124 (13)		27 (2.9)		81 (8.7)		81 (13)		15 (2.5)		34 (5.6)	
Omega-3 fatty acids[b]	101 (11)		6 (0.6)		43 (4.6)		54 (8.9)		7 (1.2)		23 (3.8)	

[a]Energy-adjusted with the residual method[21,22].

[b]Fish oil.

The number of URTI events per subject ranged from 0-8 with a mean of 0.87. Women reported more URTI events than men, 1.02 vs. 0.71, which could also be seen in the smoothed hazard estimates of URTI events/person-week in **Figure 2**. The URTI incidence peaked in September-October followed by a decline and increased again in mid-December to reach a second peak in mid-February. The lowest URTI incidence was detected in May at the end of follow-up. According to the Swedish Institute for Communicable Disease Control the incidence pattern of URTI in the Stockholm County (113 km from where the study was conducted) was similar with its first peak in October and a second peak in February[25].

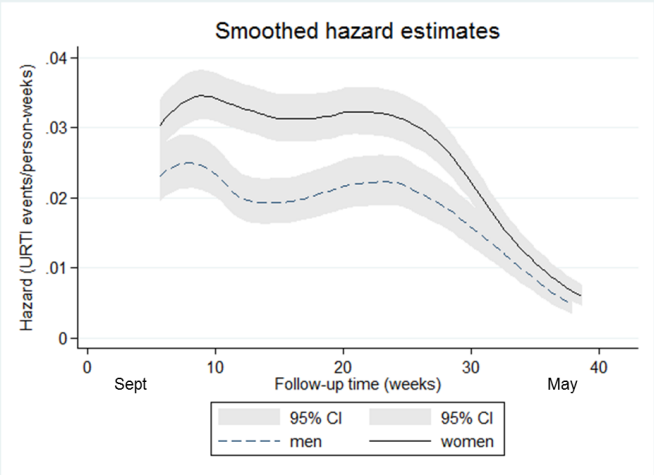

Figure 2 Smoothed hazard estimates (URTI events/person-week) for women (solid line) and men (dotted line) with 95% confidence intervals.

Table 3 shows the nutrient intake from food, the number of cases/1000 person-weeks and IRR (95% CI) for URTI among all subjects. Multivariate adjusted models (IRR (95% CI)) for women showed a reduced risk of URTI for high intake of vitamin C (0.69 (0.55-0.88)), vitamin E (0.77 (0.62-0.96)) and DHA (0.57 (0.39-0.83)), a borderline protective association for selenium (0.78 (0.61-1.01)) and AA (0.80 (0.65-0.99)), while no significant protective association for zinc. High intakes of omega-6 and EPA among women showed lower IRR compared to low intake, although the association was not statistically significant. No protective association for men could be seen for any nutrient. On the contrary, a higher risk for URTI among men was detected for medium intake of vitamin E (1.42 (1.09-1.85)) as well as for medium (1.47 (1.07-2.01)) and high (1.50 (1.04-2.16)) zinc intake.

The proportional hazards assumption was met for all models. Further, effect measure modification or interaction with time could not be found for any nutrient. Since pollen allergy can mimic URTI symptoms, we considered a model excluding subjects with pollen allergy. For vitamin C, the results were similar for women but became stronger for men when comparing high intake to low intake. For vitamin E, the results became non-significant for women comparing high to low intake as well as for men comparing medium to low intake. For AA, the result for women comparing high to low intake became non-significant, while no major changes were seen for men. For selenium, zinc, omega-6, omega-3, EPA and DHA, the results were similar for both women and men. The time point of filling out MiniMeal-Q could have affect the reported dietary intake, e.g. intake of fruit, vegetables, fish and seafood, which could depend on season. We therefore adjusted for when subjects had filled out MiniMeal-Q, however the estimates did not change for any of the nutrients.

Table 3. Intake of nutrients[a] from food and risk of upper respiratory tract infection among all subjects (n=1533).

	Crude			Crude model[b]		Multivariate model[c,d]	
	Cases	Person-weeks	Cases/1000 person-weeks	IRR	95% CI	IRR	95% CI
Intake of vitamin C (mg/d)							
Women							
<45	196	16568	12	1.00	-	1.00	-
45-110	612	49859	12	1.06	0.90-1.24	1.04	0.86-1.24
≥110	142	13718	10	0.73	0.59-0.91	0.69	0.55-0.88
Men							
<40	101	10980	9	1.00	-	1.00	-
40-100	262	25469	10	1.21	0.96-1.52	1.19	0.92-1.53
≥100	65	8039	8	0.80	0.59-1.10	0.81	0.59-1.12
Intake of vitamin E (mg/d)							
Women							
<6.5	222	18772	12	1.00	-	1.00	-
6.5-9.5	529	43453	12	1.03	0.88-1.21	0.99	0.84-1.17
≥9.5	199	17920	11	0.83	0.69-1.01	0.77	0.62-0.96
Men							
<6	73	8798	8	1.00	-	1.00	-
6-8.5	268	26240	10	1.39	1.07-1.80	1.42	1.09-1.85
≥8.5	87	9451	9	1.22	0.90-1.67	1.26	0.91-1.74
Intake of selenium (µg/d)							
Women							
<25	191	15483	12	1.00	-	1.00	-
25-45	593	49304	12	0.98	0.83-1.16	1.03	0.84-1.26
≥45	166	15358	11	0.76	0.62-0.94	0.78	0.61-1.01
Men							
<25	102	10831	9	1.00	-	1.00	-
25-40	229	22648	10	1.13	0.89-1.42	1.15	0.87-1.52
≥40	97	11009	9	0.90	0.68-1.18	0.94	0.67-1.30
Intake of zinc (mg/d)							
Women							
<7.5	186	15571	12	1.00	-	1.00	-
7.5-11	612	50308	12	0.99	0.84-1.17	1.05	0.85-1.31

≥11	152	14266	11	0.75	0.61-0.93	0.88	0.66-1.17
Men							
<7	59	7319	8	1.00	-	1.00	-
7-10	255	25297	10	1.40	1.05-1.85	1.47	1.07-2.01
≥10	114	11872	10	1.30	0.95-1.77	1.50	1.04-2.16
Intake of omega-6 (g/d)							
Women							
<0.45	204	16966	12	1.00	-	1.00	-
0.45-1.25	532	44912	12	0.95	0.81-1.12	0.93	0.78-1.11
≥1.25	214	18267	12	0.93	0.77-1.13	0.87	0.70-1.07
Men							
<0.4	68	7926	9	1.00	-	1.00	-
0.4-1.2	265	26393	10	1.30	1.00-1.70	1.26	0.94-1.71
≥1.2	95	10169	9	1.16	0.85-1.58	1.12	0.79-1.58
Intake of arachidonic acid (mg/d)							
Women							
<40	245	20077	12	1.00	-	1.00	-
40-85	522	43924	12	0.93	0.80-1.08	0.95	0.80-1.12
≥85	183	16144	11	0.81	0.67-0.99	0.80	0.65-0.99
Men							
<40	92	9943	9	1.00	-	1.00	-
40-80	249	25075	10	1.12	0.88-1.42	1.11	0.86-1.44
≥80	87	9470	9	0.99	0.74-1.32	0.96	0.70-1.32
Intake of omega-3 (g/d)							
Women							
<0.13	152	13526	11	1.00	-	1.00	-
0.13-0.5	606	50668	12	1.13	0.94-1.35	1.11	0.91-1.34
≥0.5	192	15951	12	1.11	0.90-1.38	1.01	0.80-1.29
Men							
<0.13	92	9812	9	1.00	-	1.00	-
0.13-0.45	256	25495	10	1.04	0.82-1.32	1.05	0.80-1.39
≥0.45	80	9182	9	0.84	0.63-1.13	0.83	0.58-1.18
Intake of EPA (mg/d)							
Women							
<30	221	18411	12	1.00	-	1.00	-
30-100	498	42278	12	0.95	0.80-1.12	0.95	0.80-1.13
≥100	231	19456	12	0.90	0.74-1.09	0.90	0.73-1.10
Men							
<25	80	9208	9	1.00	-	1.00	-
25-100	254	24991	10	1.26	0.98-1.63	1.26	0.95-1.68
≥100	94	10289	9	1.04	0.77-1.40	1.04	0.75-1.44
Intake of DHA (g/d)							
Women							
<0.1	193	15539	12	1.00	-	1.00	-
0.1-0.32	556	46989	12	0.93	0.79-1.09	0.81	0.61-1.07
≥0.32	201	17617	11	0.81	0.67-0.99	0.57	0.39-0.83
Men							
<0.1	80	9086	9	1.00	-	1.00	-
0.1-0.35	270	26739	10	1.23	0.96-1.57	1.06	0.76-1.49
≥0.35	78	8664	9	1.02	0.75-1.39	0.71	0.45-1.11

[a]Energy-adjusted with the residual method[21,22].
[b]n=1533
[c]All multivariate models were adjusted for age, sex, BMI, education and energy intake. Zinc was additionally adjusted for dietary selenium intake and DHA was additionally adjusted for dietary EPA intake.
[d]n=1459, 74 subjects with missing values, whereof 62 women and 12 men.

Table 4 shows the daily or weekly supplement use of vitamin C and/or multivitamin/mineral as well as omega-3, the number of cases/1000 person-weeks and IRR (95% CI) for URTI. None of the supplements showed an association with URTI incidence. The estimates were similar when excluding subjects with pollen allergy as well as when adjusting for time point of filling out MiniMeal-Q.

Table 4. Daily or weekly[a] supplement use and risk of upper respiratory tract infection among all subjects.

	Crude			Crude model[b]		Multivariate model[c,d]	
	Cases	Person-weeks	Cases/1000 person-weeks	IRR	95% CI	IRR	95% CI
Vitamin C and/or multivitamin/mineral							
All							
No	1130	103599	11	1.00	-	1.00	-
Yes	240	21035	11	1.10	0.96-1.27	1.09	0.92-1.28
Women							
No	777	66421	12	1.00	-	1.00	-
Yes	165	13724	12	1.07	0.90-1.26	1.07	0.88-1.30
Men							
No	353	37178	9	1.00	-	1.00	-
Yes	75	7311	10	1.15	0.90-1.48	1.12	0.85-1.48
Omega-3							
All							
No	1226	111724	11	1.00	-	1.00	-
Yes	144	12910	11	1.04	0.88-1.24	1.07	0.89-1.29
Women							
No	835	71178	12	1.00	-	1.00	-
Yes	107	8967	12	1.05	0.86-1.28	1.11	0.88-1.39
Men							
No	391	40546	10	1.00	-	1.00	-
Yes	37	3943	9	0.96	0.69-1.35	0.98	0.71-1.34

[a]Daily/weekly supplement intake was compared to monthly/in period/no intake.
[b]n=1533
[c]All multivariate models were adjusted for sex, age, BMI and education.
[d]n=1459, 74 subjects with missing values, whereof 62 women and 12 men.

We evaluated the dose-response relationship between dietary intake and URTI graphically with restricted cubic spline models while controlling for confounding factors. **Figures 3** and **4** illustrate the graphs for vitamin C and E, selenium and DHA depicted separately for women and men. All graphs confirmed the results seen in the categorical analyses (Table 3). The spline models for zinc, omega-6, AA, omega-3 and EPA also showed similar results to the categorical analyses (**Appendix 1**). Although not statistically significant, the spline models revealed trends of a

decreasing risk among men with increasing intakes of vitamin C, omega-6, omega-3, DHA and EPA. The same could be seen among women for increasing selenium, omega-6, AA, omega-3 and EPA intakes.

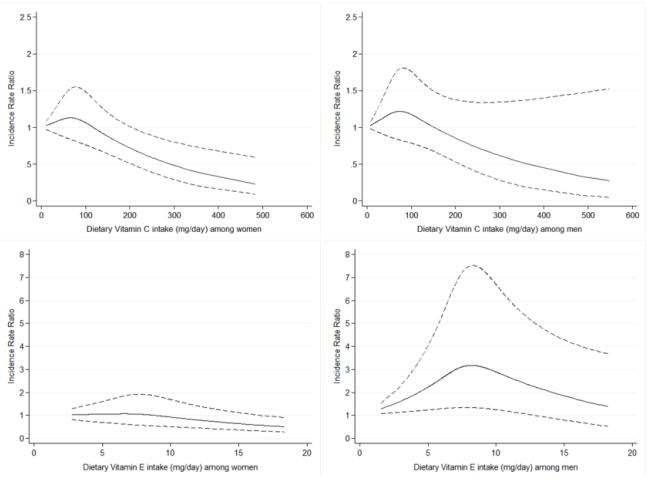

Figure 3 Restricted cubic spline models with smoothed incidence rate ratios (solid line) of URTI for dietary vitamin C and E and 95% confidence intervals (dotted lines) displayed separately for women and men. Both models were adjusted for age, energy, BMI and education. The x-axis was truncated showing 5 to 95 percentile of intake.

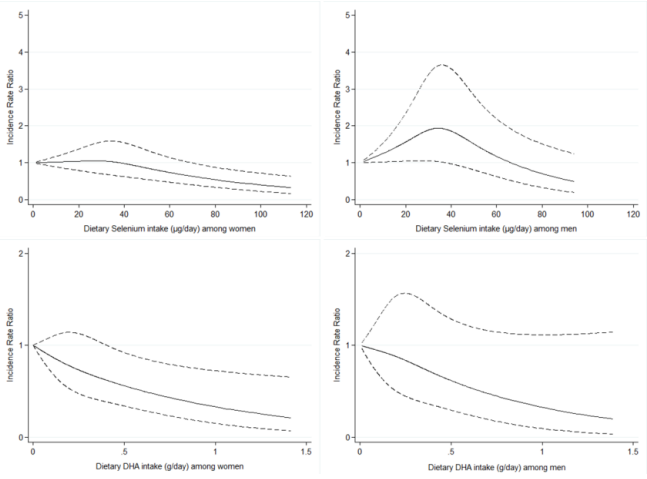

Figure 4 Restricted cubic spline models with smoothed incidence rate ratios (solid line) of URTI for dietary selenium and DHA and 95% confidence intervals (dotted lines) displayed separately for women and men. Both models were adjusted for age, energy, BMI and education. DHA was additionally adjusted for dietary EPA intake. The x-axis was truncated showing 5 to 95 percentile of intake.

DISCUSSION

This study showed that high dietary intake of vitamin C, vitamin E and DHA was associated with a reduced risk of URTI among women. There was also a suggestive protective effect for high intake of AA. No decreased risk could be seen among men, instead we found an increased risk of URTI associated with medium Vitamin E and high zinc intake from diet. We could not see any association between supplement intake and URTI among either women or men.

The present study is one of the few prospective cohort studies on dietary intake and URTI in an adult healthy population[13,14]. Other studies have mostly been randomized controlled trials conducted in specific populations. A previous cohort study from our research group examined the association between dietary and supplement intake of vitamin C and E and URTI in a Swedish cohort of 1,509 adults[14]. Similar to the present study, high dietary vitamin C intake among women was found to be protective, yet no association was seen among men. High dietary vitamin E intake showed no association among either women or men. However, among men there was a protective effect of vitamin E supplement as well as a potential beneficial effect of vitamin C supplement, while no association of any supplement could be seen among women. In a study among 4,272 Spanish university employees, neither combined dietary and supplement intake of vitamin C nor zinc was related to the incidence of colds[13]. The FFQ was however designed to assess vitamin C and zinc intake only, thus it was not possible to adjust for other nutrients (except alcohol). One can therefore not rule out the possibility that other dietary factors might have affected the URTI incidence.

In a recent meta-analysis of 29 randomized controlled trials on vitamin C the risk reduction in the general population was quite modest (relative risk: 0.97 (CI 0.94-1.00)), but both duration and severity of symptoms was decreased[6]. Vitamin C is found in high amounts in leukocytes where its concentration has been seen to rapidly decrease during an infection[26,27]. It is not fully understood how vitamin C enhances the immune function, however immune cells are particularly sensitive to oxidative stress and the vitamin's antioxidative effect offers protection to their structure and function[5]. The trials on vitamin E have mainly included elderly (≥60 y) and while one study showed protective effects[8], another study saw no effects but increased severity[7]. In a Finnish intervention study among smoking men, the effect of vitamin E supplementation among older men (≥72 years) was found to differ depending on residency and smoking[9]. We could not find any effect measure modification between vitamin E and either smoking or age in our study. However, the subjects were not as old and the proportion of smokers was rather low. Vitamin E protects cell membranes from oxidative damage and also gives resistance to infections by affecting different immune processes[26]. However, mega doses of vitamin E (approximately 300 mg/day) in human and animal cell studies have shown to impair the immune system[28,29]. Furthermore, supplement intake of vitamin E has shown to increase mortality[30]. In the

light of these studies, caution should be taken in recommending supplement intake. Zinc supplementation and URTI has been studied in several trials, whereof two of them showed a decreased incidence among children[31,32] and 14 studies a reduction in duration[12]. In our study, we could not see any association with URTI among women, and contrary to the trials we saw an increased incidence among men. In-vitro studies have showed that zinc can inhibit growth of eight of nine strains of rhinoviruses[33]. Nevertheless, high zinc levels may act pro-oxidative[27] and routine supplementation has shown adverse effects on the immune system[34].

Comparisons between the present cohort study and previous trials are not trivial. We assessed a potential association between habitual dietary intake and URTI, whereas trials intervene with supplements at higher doses than what can normally be obtained from food. The bioavailability and kinetics of a nutrient is also different depending on if originating from supplements or food[35]. An advantage with a trial is that the ordinated nutrient content from supplements is known. However, intake of other nutrients might be more difficult to assess and control for unless a rigid dietary assessment is made throughout the intervention period. Problems of adherence and dropout are yet other issues inherent in trials.

The selenium content in crops varies a lot depending on country of origin. Therefore, assessment of selenium from food can be problematic and offers one potential explanation for the lack of an association in this study. To our knowledge, there are no previous cohort studies or trials on selenium intake and URTI. Yet, selenium is a potent antioxidant that plays a crucial role in keeping the redox balance of cells[26] and animal and human cells studies have showed selenium status to affect influenza susceptibility[10,36]. Selenium is required for the activity of the antioxidative enzyme glutathione peroxidase that scavenges ROS and thereby protects cells in the immune system. The mineral itself also enhances the resistance to infection through modulation of the Th1/Th2 response[26]. Nevertheless, an excessive intake of selenium has been shown to harm the immune system[37].

To our knowledge, the present study is the first investigating the association between PUFA intake and URTI and we found DHA and possibly also AA to have protective effects among women. Eicosanoids from AA are pro-inflammatory[16,17], while resolvins from n-3 fatty acids act anti-inflammatory[15]. However, exactly how they affect the susceptibility to URTI is still to be clarified, thus further investigations are needed.

We found major gender differences in the results, which cannot merely be due to higher intakes of certain nutrients among women. Instead, there might be biological differences in nutrient metabolism and susceptibility to URTI. For reasons not well understood, women seem to have a stronger immune response than men[38,39]. Moreover, steroid hormones have been found to play a role in the immune system[40] where estrogens are associated with inflammation, whereas androgens act anti-inflammatory[41-43]. Furthermore, gender has been found to

influence the reaction to respiratory tract infections[40] and adult women experience URTI more frequently than men[44], a feature also seen in our study. Whether social behavior is a confounder here is still not clear though. A review of 51 studies showed that intake of AA and DHA among women contributed significantly more to their content in plasma lipids than among men[45]. Even if the intake of AA and DHA was similar between the sexes in our study, this gender difference in nutritional metabolism might be an explanation for the different results.

This study has some limitations that ought to be acknowledged. URTI was self-reported and not validated with an objective method, hence misclassification is possible. However, we controlled for both pollen allergy and smoking, which can mimic URTI symptoms. When controlling for pollen allergy, we saw slight differences for some nutrients indicating that it might have had an effect on URTI classification. Under-reporting of URTI events, as documented in a previous study[46], might have underestimated the effect of nutrients on URTI incidence. Also, we did not have data on URTI duration or severity, which would have been valuable to evaluate. Assessment of dietary intake should preferably have been done prospectively at baseline to prevent URTI events from influencing the assessment, potentially resulting in reverse causation. Yet, a majority (80%) of the subjects filled out MiniMeal-Q within the first five months of the study. We further believe that the public awareness of the effects of nutrients on URTI mainly concerns vitamin C. Hence, since we did not observe an increased risk of URTI with high vitamin C intakes, we consider the potential problem of reverse causation in the present study as negligible. The correlation coefficients for vitamin C and E, selenium, zinc and PUFA from the validation study of MiniMeal-Q[18,19] indicate that the nutrients were measured with some error. Nevertheless, the error ought to be non-differential with regard to URTI and would thus only attenuate the estimates. Although the intake ranges of nutrients were wide in this study, there were few subjects at high intakes, which could have influenced our ability to observe a potential association with URTI. A strength of the study is that we had the opportunity to follow our subjects over a long period to cover the general Swedish URTI season. Moreover, we evaluated a large number of potential confounding factors between dietary intake and URTI. However, it is still possible that the results might have been affected by unmeasured confounding, i.e. other dietary or supplement sources of the nutrients as well as stress, which has been shown to affect the susceptibility to URTI[47-49]. Potential residual confounding from measurement error in confounding variables could have influenced the results. Nevertheless, the overall effect of residual confounding ought to be minor since we only found a few confounders.

CONCLUSIONS

This study showed that high dietary intake of vitamin C, vitamin E, DHA and potentially also AA, were associated with a reduced risk of URTI among women. Among men, we found that medium vitamin

E and high zinc intake was associated with increased risk of URTI, while no effect could be seen for other nutrients. Supplement intake was not associated with URTI incidence among either women or men. If the protective associations found in this study hold true, the individual burden and societal economic costs due to URTI could be reduced. However, more studies on dietary intake and URTI are needed to evaluate the reproducibility of our findings.

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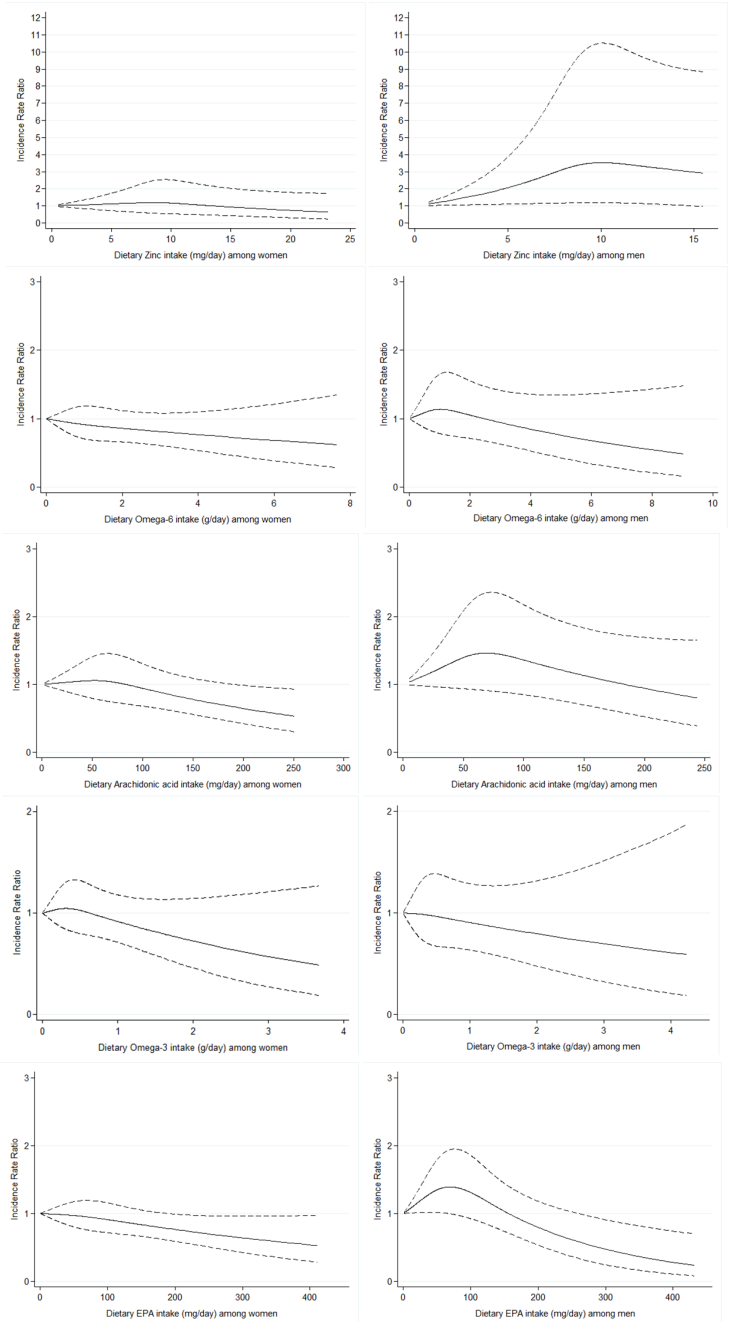
APPENDIX 1



Appendix 1 Restricted cubic spline models with smoothed incidence rate ratios (solid line) of URTI for dietary zinc, omega-6, AA, omega-3 and EPA and 95% confidence intervals (dotted lines) displayed separately for women and men. Both models were adjusted for age, energy, BMI and education. Zinc was additionally adjusted for dietary selenium intake. The x-axis was truncated showing 5 to 95 percentile of intake.

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